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OF THE

# BUREAU OF STANDARDS

S. W. STRATTON, DIRECTOR

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No. 375

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THE DOUBLE-POLARIZATION METHOD FOR ESTI-  
MATION OF SUCROSE

AND

THE EVALUATION OF THE CLERGET DIVISOR

BY

RICHARD F. JACKSON, Associate Chemist

and

CLARA L. GILLIS, Assistant Chemist

*Bureau of Standards*

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MARCH 30, 1920



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# THE DOUBLE-POLARIZATION METHOD FOR ESTIMATION OF SUCROSE AND THE EVALUATION OF THE CLERGET DIVISOR

By Richard F. Jackson and Clara L. Gillis

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## I. INTRODUCTORY

### 1. GENERAL STATEMENT

An accurate system of sugar analysis, in which the quartz-wedge saccharimeter is employed, depends, primarily, upon the establishment of the fundamental constants and upon a rigorous specification of the procedure to be followed in operations where these constants are involved. Next in importance to the constants which form the basis of the saccharimeter scale—namely, the rotation of pure sucrose and its quartz equivalent in terms of monochromatic light—is that which enables us to estimate the sucrose content in a mixture containing other optically active constituents. This latter is known as the Clerget constant. The nature of this constant is, briefly, as follows: The polariscopic reading of a solution is the resultant rotation of all optically active substances present. If sucrose alone is present, the reading, if made under standard conditions, is an exact measure of this sugar. If, together with sucrose, other sugars are present, the first polariscopic reading is not a measure of the sucrose, but must be supplemented by a second, in which the rotation of the other sugars is kept constant, while that of sucrose is subjected to a change which can be measured and is known to be an exact function of the quantity of sucrose. This change is most easily and exactly brought about by hydrolysis. By this hydrolysis or “inversion” the sucrose molecule is split, with the addition of 1 molecule of water, into



2 molecules of simpler sugars, the one d-glucose or dextrose, the other d-fructose or levulose. It is upon the ease with which this split occurs that the classic method of Clerget depends.

If  $D$  represents the change in polariscopic reading before and after inversion and  $t$  the centigrade temperature, the per cent of sucrose is given by the formula

$$S = \frac{100 D}{C - at} \quad (1)$$

in which  $C$  is the Clerget constant and  $a$  its temperature coefficient. The constant obviously represents the change in rotation of pure sucrose before and after inversion.

The hydrolysis for analytical purposes may be brought about by either of two catalytic agents, namely, the enzyme invertase or hydrochloric acid. Invertase, from the standpoint of accuracy and the avoidance of disturbing side reactions, is the ideal hydrolyzing agent. It is perfectly selective in its action upon the compound sugars of the sucrose group. It is, however, a laborious and difficult substance to prepare properly, and its hydrolyzing action is too slow for routine analytical work.

The methods in which hydrochloric acid is used are the ones most widely employed and, in fact, are the only ones which are feasible when the operator's time must be conserved. There are, however, many difficulties and sources of error which apparently are inherent in these methods, but which, owing to the greater simplicity of the analysis, are, in general, tolerated. It was the purpose of this research to examine these sources of error and inquire which could be eliminated, in the hope that the method of acid inversion might be made to approach that of the invertase inversion in respect of accuracy and reliability. The present paper, therefore, describes the results of a study of the acid method of inversion.

## 2. HISTORICAL

The literature relative to the Clerget analysis is too voluminous to permit any but a very general survey. It is probable that the contributions describing researches on this subject number over a hundred. The net results of this work are summarized and discussed critically by Browne<sup>1</sup> and do not require reproduction here. In the following review only the few researches which are particularly pertinent to the present paper are briefly described.

---

<sup>1</sup> Handbook of Sugar Analysis, chap. 10, p. 263.

In 1849 Clerget<sup>2</sup> proposed the double-polarization method for the determination of sucrose in the presence of other optically active substances. He took 50 ml<sup>3</sup> of a normal solution in a 50 to 55 ml flask and filled it to the 55 ml mark with "pure and fuming" hydrochloric acid. After shaking, he placed the mixture in a water bath the temperature of which was so regulated that 10 minutes were required to bring the solution to 68° C. Upon attaining temperature, the flask was removed from the bath, cooled rapidly to 20°, and the solution polarized. The invert reading was multiplied by 11/10. The per cent sucrose was calculated from the formula

$$S = \frac{100 D}{144 - \frac{t}{2}} \quad (2)$$

where  $D$  is the algebraic difference between the two corrected polarizations, and  $t$  the centigrade temperature.

In modifying Clerget's method, Herzfeld<sup>4</sup> took the half-normal weight of sugar (13 g) in 75 ml of solution and added 5 ml of hydrochloric acid (38 per cent, or 1.188 specific gravity). After shaking, he immersed the flask in a water bath and warmed the contents to 67 to 70°. Herzfeld prescribed that this should be accomplished in from two to three minutes. The solution having attained temperature, he kept it as near 69° as possible for another five minutes, when it was quickly cooled, made to a volume of 100 ml at 20°, and polarized at the same temperature. Being a half-normal solution, the reading was multiplied by 2. Under these conditions the Clerget formula becomes

$$S = \frac{100 D}{142.66 - 0.5 t} \quad (3)$$

Browne<sup>5</sup> has modified this formula, making it applicable to concentrations other than 13 g of sucrose in 100 ml. As modified, it is

$$S = \frac{100 D}{142.66 - \frac{t}{2} - 0.0065 (142.66 - \frac{t}{2} - D)} \quad (4)$$

<sup>2</sup> Ann. Chim. Phys. (3), 26, p. 175; 1849.

<sup>3</sup> ml signifies the milliliter, which is one-thousandth of a liter.

<sup>4</sup> Zeit. Ver. Zuckerind., 38, p. 699; 1888.

<sup>5</sup> Jour. Assoc. Off. Agr. Chem., 2, p. 134; 1916.

In order to obviate multiplying the error of reading by 2, Browne advocated a return to the original Clerget procedure, slightly modified. He added 5 ml of concentrated hydrochloric acid to 50 ml of normal sugar solution in a 50 to 55 ml flask, and allowed the solution to stand overnight at room temperature, when it was made to volume and read at 20° C. By adjusting the volume after inversion, the error inherent in Clerget's method was eliminated. This error was caused by a diminution in volume during inversion and amounted to about one-third of a milliliter in 55 ml. Browne used the following formula:

$$S = \frac{100 D}{144.9 - \frac{t}{2}} \quad (5)$$

which, when corrected for differences in specific rotation of invert sugar with varying concentration, becomes

$$S = \frac{100 D}{144.9 - \frac{t}{2} - 0.01 (144.9 - \frac{t}{2} - D)} \quad (6)$$

A simplification in the method of performing the inversion was suggested by Walker.<sup>6</sup> He took 50 or 75 ml of the solution prepared for the direct polarization in a 100 ml flask (if 50 ml, 25 ml of water was added), heated the solution in a water bath to 65° C, removed it from the bath, and added 10 ml of a mixture of equal volumes of hydrochloric acid (specific gravity 1.188) and water. The flask was allowed to stand 15 minutes or longer at room temperature before making to volume at 20° C. He polarized the solution at 20° C, multiplying the reading by the proper concentration factor.

A method of inversion at room temperature with increased acid strength was suggested by Steuerwald.<sup>7</sup> He measured 50 ml of normal sugar solution into a 100 ml flask. To this he added 30 ml of hydrochloric acid (1.1 specific gravity) and allowed the solution to stand 3 hours, if the temperature was between 20 and 25° C, or 2 hours if above 25°. The volume was then adjusted and the solution polarized at 20°. The value of the Clerget constant under these conditions became 145.53. The variations in the constant with concentration of sugar were assembled in an accompanying table.

<sup>6</sup> Jour. Ind. Eng. Chem., 9, p. 490; 1914.

<sup>7</sup> Int. Sug. Jour., 15, p. 489; 1913.

Of the various modifications of Clerget's method the one most widely used is Herzfeld's. He established 142.66 as the Clerget constant, a value which, though frequently found too low, is still in general use. At the New York meeting (1912) of the International Commission for Uniform Methods of Sugar Analysis, Herzfeld<sup>8</sup> requested that this value be retested.

### 3. PLAN AND OBJECT OF PRESENT RESEARCH

Our purpose during the present investigation has been to ascertain the conditions essential to the use of the Clerget method. The essentials are a knowledge of the change in rotation which sucrose undergoes on inversion and the assurance that the rotation of every substance in solution except sucrose remains constant.

The research has divided itself, broadly, into five divisions. In preliminary experiments we have studied the interrelation of time, temperature, concentration of acid, and velocity of inversion, and thus have been able to determine the time at each temperature and acidity necessary for complete inversion. These reaction velocities have been determined for various constant temperatures and subsequently for rising and for falling temperatures. Moreover, we have measured the rate of decomposition in the presence of the catalyzing acid after the completion of the inversion.

Secondly. We have determined with as great precision as possible the value of the rotation of invert sugar inverted by acid of the concentration prescribed by the prevailing methods. The inversion has been conducted in such a way that the reaction is complete and subsequent decomposition avoided.

Thirdly. We have ascertained what modifications in procedure are necessary when the sample to be analyzed contains impurities which may undergo a change in rotation in the presence of the catalyzing acid.

Fourthly. We have determined the influence of a change in concentration of acid upon the rotation of invert sugar and the effect of neutralization of the acid after completion of the inversion, and have endeavored to correlate the various values of the Clerget constant and the various proposed modifications in method.

Finally. We have proposed specifications for a correct procedure and have ascertained with what precision known mixtures may be analyzed by the methods proposed.

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<sup>8</sup> Bureau of Standards Circular No. 44, p. 189.

## II. PRELIMINARY EXPERIMENTS

## 1. VELOCITY OF INVERSION OF SUCROSE AND ITS TEMPERATURE COEFFICIENT

The velocity of inversion of sucrose has been the subject of numerous investigations and the basis of much discussion in the field of homogeneous catalysis. While the present research was undertaken with the purpose of suggesting improvements in analytical methods, the results, or at least the methods of measurement proposed, should be adaptable to any purpose involving the phenomena of reaction velocity.

In every instance in which the velocity has been studied previously, the method has been to follow the progress of the inversion on the polarimeter by successive readings of the instrument at measured intervals of time. The formula

$$k = \frac{1}{t} \ln \frac{R_0 - R_\infty}{R_t - R_\infty} \quad (7)$$

in which  $k$  is the velocity constant,  $t$  the time in minutes,  $R_0$  the initial rotation,  $R_\infty$  the rotation after complete inversion, and  $R_t$  the rotation at the time  $t$ , gives a satisfactory constancy for  $k$ , provided, as Rosanoff<sup>9</sup> has shown, a proper value is assigned to  $R_\infty$ . Rosanoff has shown that the reaction obeys precisely the unimolecular formula. Arrhenius<sup>10</sup> very early studied the effect of temperature on the velocity and showed that kinetic considerations were entirely inadequate to explain the enormous temperature coefficient. The increase in kinetic energy corresponding to a 10° rise in temperature might be expected to cause an increase of 1 or 2 per cent in the observed velocity, whereas we get an effect about 200 times as great. Arrhenius proposed the hypothesis that some sugar molecules were active and could suffer inversion, while others were inactive. Inactive molecules were converted into active ones by hydration or by some tautomeric or similar change. This involved a chemical equilibrium with its constant

$$k = \frac{(\text{active molecules})}{(\text{inactive molecules})} \quad (8)$$

If this is an ordinary chemical equilibrium, the displacement of its constant with temperature follows the equation of van't Hoff:

$$\frac{d \log k}{dt} = \frac{Q}{RT^2} \quad (9)$$

<sup>9</sup> J. Amer. Chem. Soc., 23, p. 1912; 1911.

<sup>10</sup> Zeit. physik. Chem., 4, p. 230; 1889.

which may be integrated to the equation

$$k_{T_2} = k_{T_1} e^{\frac{Q}{R} \left( \frac{T_2 - T_1}{T_2 T_1} \right)}, \quad (10)$$

in which  $Q$  represents the heat of reaction between inactive and active molecules,  $k$  the equilibrium constant between them,  $R$  the gas constant, and  $T$  the absolute temperature. Arrhenius further assumes that active molecules are relatively small in number and that the reaction velocities are proportional to their concentration. The velocities of the reaction may then be expressed by equation (10), in which  $k$  now represents the velocity constants instead of the equilibrium constants.

Fanciful as the assumption may seem, the fact is that the equation fits the experimentally determined values of  $k_T$  over a series of temperatures extending to 55° C. No measurement at higher temperature than this has previously been attempted.

Later thinkers have given a more plausible basis for the fundamental equation (10). Marcelin<sup>11</sup> proposed that a molecule reacted when its internal energy reached a certain minimum value. Rice<sup>12</sup> calculated the probability that a molecule would possess this energy. W. C. McC. Lewis<sup>13</sup> suggested that this energy was derived from the infra-red radiation which was present in the system. These authors derive collectively and individually the formula

$$k_{T_2} = k_{T_1} e^{\frac{E}{R} \left( \frac{T_2 - T_1}{T_1 T_2} \right)}, \quad (11)$$

where  $E$  is the "critical increment" of energy required to make a molecule react. This formula is of the same form as the van't Hoff equation and leads to the same integrated formula as that which Arrhenius derived. This integration demands that  $E$  or  $Q$  be constant within the limits of the integration. If the formula is to be used, this point should be verified experimentally. To do this was one of the objects of the present investigation.

All previous experiments have been performed within a very limited range of conditions, namely, those dictated by ordinary laboratory convenience. The method in which the inversion is followed on the polarimeter necessitates that the reaction be relatively slow and precludes any considerable range of temperature unless great precautions are taken to insure its constancy. For extending the range of both velocity and temperature, the method

<sup>11</sup> *Compt. rend.*, 158, p. 161.

<sup>12</sup> *J. Chem. Soc.*, 107, I, p. 266; 1915.

<sup>13</sup> *J. Chem. Soc.*, 105, p. 2330, 1913; 107, p. 233, 1915; 109, p. 55, 1917; 109, p. 67, 1917; 109, p. 796, 1917.



of carrying on the reaction in the polariscope tube was deemed unsuitable. The one described below was substituted.

In the method used here the reaction was started by mixing the reacting liquids quickly, and when about half complete was abruptly arrested by adding a quantity of alkali sufficient exactly to neutralize the acid which catalyzed the inversion.

The reaction was carried out in long-necked flasks of about 100 ml content. The neck was 14 cm in length and 25 mm in diameter. In preparation for a measurement, the sugar solution was placed in the flask and the acid in a test tube which passed through a rubber stopper and dipped into the sugar solution. The rubber stopper closed the flask, the test tube being even with its top surface. The tube was closed by a rubber stopper, through which passed a glass rod extending nearly to the bottom of the test tube. The whole apparatus was immersed in the liquid of a thermostat up to the rim of the flask and allowed to take the temperature of the bath. When the temperature had attained equilibrium, the glass rod was plunged violently through the bottom of the test tube, releasing the acid, which, flowing from a higher level with about 5 cm head and practically no resistance to its flow, mixed almost instantaneously with the sugar solution. At the same time the flask was given a quick shake to insure thorough mixing. If the reaction was to be a rapid one, a stop watch was used to time it; otherwise the time was recorded by an ordinary watch.

When sufficient time had elapsed for about one-half decomposition of the sugar, the test tube, which had contained the acid, was quickly removed and another one inserted which contained an exactly equivalent quantity of alkali. This was broken at once by the glass rod before it could influence the temperature of the reacting mixture and the same quick action taken to insure prompt mixing of the alkali and acid. The flask was then removed from the thermostat and, if hot, was cooled by a stream of cold water and prepared for analysis. The time occupied by the process of mixing was estimated to be well within a second.

In measuring reaction velocity the condition requiring the closest control is that of temperature. Since the velocity varies 0.3 per cent to 0.4 per cent for 0.°01 change of temperature, it is obvious that a constant temperature is of the first importance. For attaining this N. S. Osborne very kindly placed at our disposal the excellent thermostat which he had used in determining the physical constants of ammonia. In this apparatus the liquid (kerosene oil) was contained in a Dewar vessel. Stirring was so

effective that the indicated temperature was independent of the location of the thermometer. The temperature fluctuation, which ranged but two or three thousandths of a degree from the mean, occurred with perfect regularity, the relay operating every seven seconds. The mean temperature was, therefore, within a few thousandths of a degree of that indicated. Temperature was measured by a calibrated platinum resistance thermometer. Preliminary experiments were made to ascertain the length of time required for the solution and acid to take the temperature of the bath.

The test tube which contained the acid was of the ordinary type. The end was softened and blown out very thin in order that the impact of the glass rod would completely shatter the entire end of the tube.

The concentration of sugar was the same in all the experiments, namely, 13 g in 55 ml at 20° C. No account was taken of the expansion of the solution at the higher temperatures, although this would change slightly the volume concentration of acid. The mass concentration remains constant. In comparison with the temperature effect, these concentration effects are vanishingly small.

In order to avoid the necessity of weighing out numerous samples of sugar and water, the relative quantities of sugar, water, and acid solution required to make up a volume of 55 ml at 20° of a solution containing 13 g sugar and 0.01 N HCl were determined by several measurements. The mixture acquired the proper concentrations if 10 ml of 0.055 N HCl was added to a solution containing 13 g of sugar and 36.790 g of water. A considerable quantity of sugar solution was made up, having this proportion of sugar and water. A pipette made by shrinking an ordinary 50 ml pipette in the blast flame was calibrated to deliver the correct weight of sugar solution, namely, 49.790 g.

After the reaction was completed and the acid neutralized, the solution was transferred through a funnel into a 100 ml flask, the reaction flask, test tubes, and glass rod being rinsed several times and all the washings added to the original solution. The volumetric flask was placed in a thermostat, the solution made to volume at 20°.00, and its rotation observed on the saccharimeter.

At any one temperature the velocity is represented by the formula

$$k = \frac{1}{t} \ln \frac{R_0 - R_\infty}{R_t - R_\infty} \quad (7)$$

$R_0$  and  $R_\infty$  for any given concentration of sugar and acid are constant. These values were determined definitely for each concentration of acid and used throughout the whole series of experiments.  $R_0$  is the rotation of 13 g of sucrose in the presence of 0.0055 N NaCl in 100 ml of solution.  $R_\infty$  is the rotation of 13 g of sucrose completely inverted by 0.01 N HCl and finally neutralized by the equivalent quantity of alkali. Each of these rotations, including  $R_t$ , is thus in a stationary state during the observation and can be read at leisure.  $R_0$  was found to be 49.996,  $R_\infty$ , -16.009; the numerator was thus the constant quantity 66.005.

In illustration of the greater adaptability of the method described above, we may cite the great extremes of velocity and compare the results with the recent work of Lamble and Lewis.<sup>14</sup> The most rapid reaction with which they experimented was conducted at 45° and was catalyzed by 0.9 N HCl. Their constant in common logarithms was 0.0647. Such were the difficulties of so rapid a reaction that their three determinations varied by about 9 per cent. By the method described above two measurements were made at 90°, one lasting 30 seconds, the other 57 seconds; the constant resulting from the one was 1.020 at 90°.32, from the other 1.033 at 90°.29. The agreement in the measurements of this reaction, which is 16 times faster than that of Lamble and Lewis, is satisfactory and indicates that the mixing of the liquid must have been very rapid, since 1 per cent variation in  $k$  may result from an error of only 0.3 to 0.5 of a second.

In Table 1 are recorded the velocities of reactions catalyzed by 0.1 N HCl. The values are in terms of common logarithms and minutes. In column 4 are given the values calculated by Arrhenius' formula (10) on page 132. The experiments at 30 and 49°.84 were used to determine the value of  $\frac{Q}{R}$ . The velocities at the remaining temperatures were then computed and, as will be seen from the table, are in agreement with the observed values within 2 or 3 per cent. The experiments at 0 and 15° are disregarded in this connection. The velocities were so low that in the time allowed the reaction had progressed but a few per cent. Hence the error of measurement was very large.

The corresponding data for 0.01 N HCl are recorded in Table 2.

A convenient method of solving these equations is by use of a table of exponential functions<sup>15</sup> in which values of  $x$  are given in

<sup>14</sup> J. Chem. Soc., 107, 1, p. 240; 1915.

<sup>15</sup> Smithsonian Physical Tables, p. 48; 1916.

the function  $e^x$ . We may conclude that the formula expresses the temperature influence as closely as can be judged from the experiments and that it is a safe instrument in devising analytical methods.

We next determined the velocities of the reaction when catalyzed by acid of the concentration prescribed in the prevailing methods of analysis. These determinations were conducted with respect to volumes of solution and concentration of acid in the manner in which the analysis itself is performed. We encountered here a difficulty which was absent in the experiments with more dilute acid. This arose from the fact that 5 ml of concentrated hydrochloric acid, even when diluted to 10 ml with water, caused by its heat of dilution a rise in temperature when added to the sugar solution. In order to overcome this, we diluted the acid in the test tube as much as the apparatus would permit and cooled it, before adding it to the sugar solution, to such a temperature that the cooling effect of the acid solution was just equal to its heat of dilution. If the sugar solution at 40° C. occupied a volume of 70 ml and the 5 ml of 38.8 per cent HCl were diluted to 10 ml, the latter required to be cooled to 22°.0 C. before its addition to the sugar solution. If the 5 ml of acid were diluted to 20 ml and the sugar solution occupied a volume of 60 ml, it was only necessary to cool the acid to 39°.32.

The measurements were made at 30 and 40° by the method described. An additional measurement was made at 20° by the usual method of carrying on the inversion in the polariscope tube and observing its progress by frequent settings of the polariscope.

The experimental data are given in Table 3. The value of the constant  $Q/R$  was found to be 13087.6. By utilizing this value and by selecting the velocity at 40° as a basis, the constants of the reaction from 20 to 70° were computed and are assembled in column 6 of Table 4. From these values the time required to invert 99.99 per cent of the sucrose present was computed from equation (7) on page 131. Similarly, the corresponding data for 0.01 N and 0.1 N HCl are given in columns 2 to 5.

TABLE 1.—Data on Reaction Velocities at Various Temperatures

[The catalyzer was 0.1 N HCl.  $R_0 = +49.96$ .  $R_\infty = -16.09$ .  $Q/R = 12925.2$ ]

Temperature, in degrees centigrade	Time	$R_t$	$k_r$ observed	$k_r$ calculated
	Minutes	$^{\circ}\text{S}$		
0.....	2571	+47.01	0.0000077	0.0000080
15.098.....	338	+35.39	.000092	.000096
30.000.....			(.0008732)	(.0008732)
39.916.....	39.916	+16.89	.00334	.00338
49.840.....	32.33	+10.83	(.01206)	(.01206)
59.903.....	10.35	+ 9.56	.04003	.04054
69.974.....	3.65	+ 7.28	.1236	.1269
80.130.....	1.583	+ 1.14	.3687	.3761
90.292.....	.677	- 2.90	1.033	1.048
90.316.....	.917	- 8.43	1.020	1.051

TABLE 2.—Data on Reaction Velocities at Various Temperatures

[The catalyzer was 0.01 N HCl.  $R_0 = +49.996$ .  $R_\infty = -16.009$ .  $Q/R = 12940.05$ ]

Temperature, in degrees centigrade	Time	$R_t$	$k_r$ observed	$k_r$ calculated
	Minutes	$^{\circ}\text{S}$		
30.00.....	2783	+23.15	0.0008148	(0.0008148)
49.85.....	226	+20.80	.001122	.001124
59.90.....	98	+12.21	.003766	(.003766)
69.97.....	31.17	+12.09	.01194	.01190
80.10.....	10.97	+11.75	.03429	.03480
90.30.....	4.08	+11.30	.0939	.0972
90.30.....	3.67	+13.84	.0939	.0972

TABLE 3.—Data on Reaction Velocities at Various Temperatures

[The catalyzer was 0.7925 N HCl.  $R_0 = +59.40$ .  $R_\infty = -20.42$ .  $Q/R = 13087.6$ ]

Temperature, in degrees centigrade	Time	$R_t$	$k_r$ observed	$k_r$ calculated
	Minutes	$^{\circ}\text{S}$		
20.....			0.002156	0.002160
30.117.....	28.05	+21.88	.00983	.00958
30.093 <sup>a</sup> .....	30.82	+18.69	.01001	.00955
35.072.....	29.08	+ 2.20	.01882	.01866
40.078.....	11.90	+ 7.92	.03780	.03783
40.088.....	11.80	+ 8.03	.03791	.03788

<sup>a</sup>In this determination  $R_0 = +69.31$  and  $R_\infty = -23.93$ .

TABLE 4.—Time Required at Various Temperatures for 99.99 Per Cent Inversion

[In the presence of 0.01 N, 0.1 N, and 0.7925 N HCl as catalyzer]

Temperature, in degrees centigrade	0.01 N HCl		0.1 N HCl		0.7925 N HCl	
	Velocity constant	Time for 99.99 per cent inversion	Velocity constant	Time for 99.99 per cent inversion	Velocity constant	Time for 99.99 per cent inversion
		Hours		Hours		Hours
20.....	0.00001899	3511	0.0002032	328	0.002161	30.8
25.....	.00003984	1673	.0004264	156	.004569	14.6
30.....	.00008148	818	.0008737	76	.009427	7.1
35.....	.0001629	409	.001747	38.2	.01900	3.5
40.....	.0003186	209	.003453	19.3	.03785	a 106
50.....	.001145	58	.01230	5.4	.1365	a 29.3
60.....	.003806	17.5	.04151	1.6	.4606	a 8.7
70.....	.01182	5.6	.1273	a 31.4	1.447	a 2.76
80.....	.03303	2.02	.3542	a 11.3		
90.....	.08982	0.74				

a Minutes.

While the above data represent the actual velocities, they should be used with caution in theoretical deduction, since it is possible that the heat of inversion in the very rapid reactions may have affected the temperature slightly. Conceivably the true velocity constants would then differ slightly from those observed.

## 2. CURVES SHOWING APPROACH TO COMPLETE INVERSION

Since the various methods of acid inversion are carried out under quite dissimilar conditions, several series of experiments were performed to observe the velocity of inversion and the time required for its completion, reproducing the conditions of the different methods as nearly as possible. In every case, 10 ml of 6.40 N HCl (equivalent to 5 ml of 38.8 per cent acid; see p. 147) was used to invert the sugar. In each series a stock sugar solution was made up and the required amount was pipetted into the reaction flasks and acidified. The inversion was abruptly stopped at the expiration of definite periods of time by the addition of a sodium hydroxide solution from a pipette delivering in a few seconds. The hydroxide had been made up exactly equivalent to the acid used as the catalyzer. The solutions were then cooled, made to volume at 20° C and polarized at the same temperature.

Since in the experiments we are interested not in the exact rotation of these solutions but merely in the time necessary to complete the reaction, we have for ready comparison multiplied



the maximum rotation in each series, except that in which Browne's modification was used, by such a factor as to make it equal  $-34.02$ . All other rotations of the series were then multiplied by the same factor. The value  $-34.02$  is twice the negative rotation of a half-normal solution of sucrose when inverted by 10 ml of 6.4 N acid and neutralized. (See p. 171.) We have plotted curves to show the relationship in the time required to to effect inversion by the different methods of procedure.

CURVE NO. 1 shows the rate at which a sugar solution approaches complete inversion when maintained at  $60^{\circ}$  throughout the whole course of reaction. The data were obtained by computing the value of  $R_t$  in equation (7) at various intervals of time when  $k=0.4606$ . The inversion is calculated to be 99.99 per cent complete in  $8\frac{1}{2}$  minutes.

CURVE NO. 2 shows the progress of the reaction when the solution is acidified at room temperature and placed in the water bath, which is regulated at  $60^{\circ}$ . A stock solution was made up to contain 15.6 g of sucrose in 70 ml of solution. A 70 ml portion was pipetted into a 100 ml flask and 10 ml of acid was added. The flask was immersed in the water thermostat and the stop watch was started. After 3 minutes, during which the solution was shaken continuously, the sodium hydroxide was added and the solution cooled and made to volume. This procedure was repeated several times with increased periods of heating. By an examination of curve No. 2, it will be seen that the reaction should be complete in 9 minutes.

CURVE NO. 3 shows the time required for complete inversion in accordance with Herzfeld's method. Fifty milliliter portions of a normal solution were diluted to 70 ml in 100 ml flasks. After the addition of 10 ml of acid, the flasks were immersed in the water bath, and the reaction was successively stopped, as in the experiments described. The bath was so regulated that the contents of the flasks reached  $69^{\circ}$  in  $4\frac{1}{4}$  minutes and remained within  $0^{\circ}.2$  of that temperature during the 5 minute period following. By shaking the flasks frequently the temperature was kept uniform. When the inversion is accomplished in this manner, the maximum value is reached in a total time of  $5\frac{1}{2}$  minutes and the polarization decreases quite noticeably on continued heating because of the decomposition of the invert sugar. The shortest time that the solution may be heated if Herzfeld's conditions are strictly adhered to is 7 minutes. In practice it is

frequently extended to as much as 10. Curve No. 3 shows that in these experiments the maximum value is obtained in  $5\frac{1}{2}$  minutes, although  $4\frac{1}{4}$  minutes were required for the temperature of the solu-

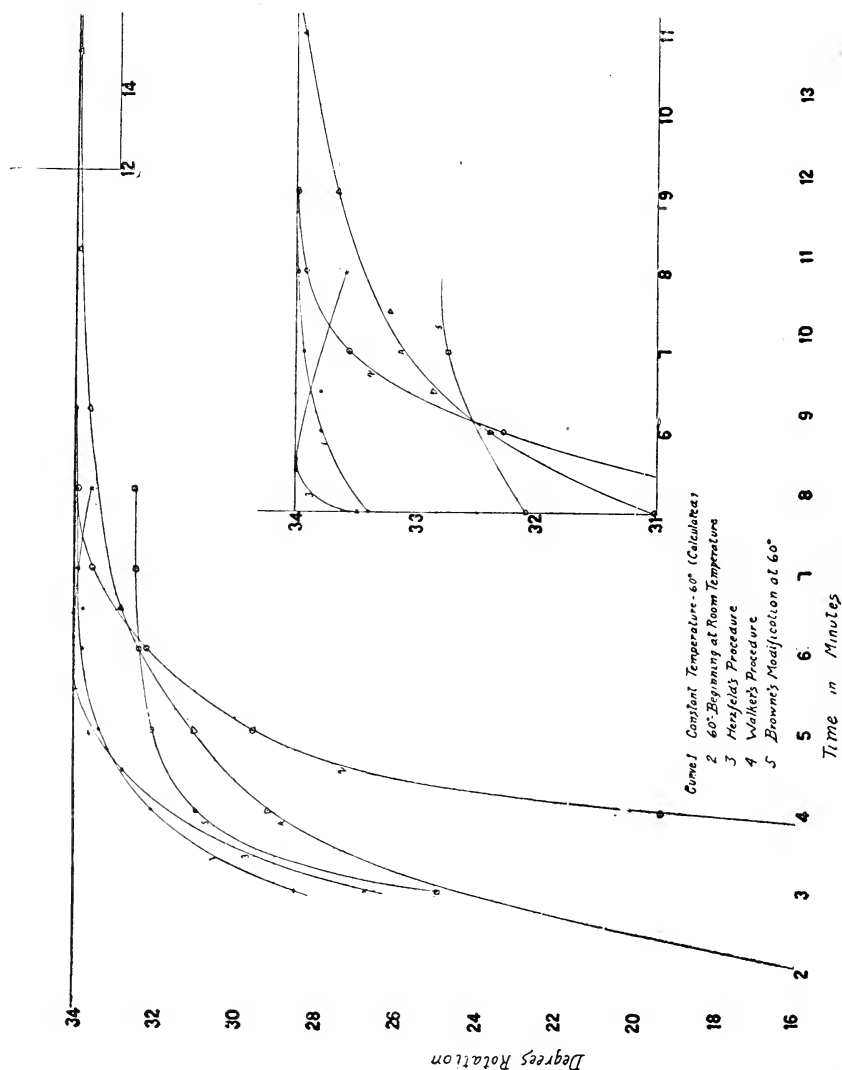


FIG. 1.—Curves showing progress of inversion of sucrose solutions under varying temperature conditions

The solution (Curves 1 to 4) is completely inverted when its rotation becomes  $-34.00$ . Note the rapid attainment of the maximum shown by Curve No. 3 and the evidence of subsequent decomposition. Curve No. 5 shows complete inversion in eight minutes.

tion to reach  $69^\circ$ . If the temperature had been brought to  $70^\circ$  in 2 minutes, as may happen in following Herzfeld's original procedure, the maximum value would have been reached in even a shorter time.

CURVE No. 4 shows the progress of the reaction when Walker's method of inversion is followed.<sup>16</sup> In this series, 50 ml portions of a normal solution were diluted in 100 ml flasks to 70 ml and heated to 65° C in the water bath. Upon attaining temperature the flasks were removed from the thermostat one by one, and 10 ml of the acid was added. The solutions were allowed to stand at room temperature for definite periods, when, as in the other series, the sodium hydroxide was added and the reaction stopped. Walker<sup>17</sup> prescribes that the solution should stand for 15 minutes before making to volume. Curve No. 4 shows that the reaction is practically complete in 12 minutes with no decomposition on continued standing. Walker found no change in rotation if the solutions are allowed to stand 30 minutes or longer.

CURVE No. 5 shows the approach to complete inversion of a solution made up with respect to concentrations of sugar and acid, as suggested by Browne in his modification of Clerget's original procedure.<sup>18</sup> Browne showed that inversion was completed if the solution was allowed to stand at room temperature overnight. The following experiments show the required time if the inversion is carried out at the temperature of 60°. The sugar solution was made up to contain the half-normal weight in 40 ml. Ten milliliters of acid was added and the flask immersed in the bath at 60°. At definite intervals of time the reaction was stopped by adding 5 ml of alkali of sufficient concentration to neutralize the acid. The addition of the strong alkali to the hot solution when there was very little room to effect quick mixing caused considerable decomposition of the invert sugar. This was evidenced by the discoloration and low rotation of the solutions. No attempt was made to eliminate this decomposition, since we were concerned only with the time required to obtain the maximum rotation. Curve No. 5 shows that a 7½ to 8 minute period is necessary. The rotations were multiplied by 11/10, but were not further corrected.

### 3. DECOMPOSITION OF INVERT SUGAR IN THE PRESENCE OF HYDROCHLORIC ACID

The data acquired in the experiments described above enable us to determine the time required under a given set of conditions to produce complete inversion of sucrose. It now becomes important to ascertain the effect of an excessive time of heating, or, in other words, the rate of decomposition of invert sugar in

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<sup>16</sup> See p. 129.<sup>17</sup> J. Ind. Eng. Chem., 9, p. 490; 1917.<sup>18</sup> See p. 129.

the presence of acid over the range of temperature commonly employed.

In performing these experiments 350 ml of solution containing the half-normal weight in 70 ml was taken for the series at each temperature. Fifty cubic centimeters of the acid was added and the acidified solution placed in the thermostat set at the desired temperature. When the inversion was complete, a sample sufficient to fill a polariscope tube was rapidly taken. This was run into a small flask, immersed in ice water, and the flask corked. Other samples were taken in the same way at carefully measured intervals of time. The polarization of these solutions were multiplied by such factors as to make the rotation of the first sample of each group equal  $-33.25$ ; that is, twice the reading of a half-normal solution when completely inverted. The rate of decomposition was calculated for each temperature.

The experimental results are assembled in Table 5. The rate of decomposition is, in common with all thermal reactions, a very large function of the temperature, a  $10^{\circ}$  rise in temperature, causing an average of 370 per cent increase in the velocity of reaction. The rates of decomposition are summarized in Table 6.

TABLE 5.—Decomposition of Invert Sugar in the Presence of 0.7925 N HCl <sup>a</sup> at Various Temperatures

80° C			70° C		
Time, in minutes	Observed reading	Reading $\times 1.605$	Time, in minutes	Observed reading	Reading $\times 1.5825$
	°S	°S		°S	°S
0.....	20.72	33.25	0.....	21.00	33.25
3.....	20.31	32.61	4.....	20.85	33.00
6.....	19.94	32.00	8.....	20.62	32.62
10.....	19.50	31.30	12.....	20.51	32.46
14.....	19.20	30.82	17.....	20.38	32.26
19.....	18.73	30.07	22.....	20.15	31.89
			32.....	19.87	31.45

60° C			50° C		
Time, in minutes	Observed reading	Reading $\times 1.575$	Time, in minutes	Observed reading	Reading $\times 1.575$
	°S	°S		°S	°S
0.....	21.11	33.25	0.....	21.11	33.25
8.....	21.02	33.11	38.....	20.98	33.04
18.....	20.90	32.93	78.....	20.92	32.95
33.....	20.80	32.76	128.....	20.83	32.80
48.....	20.70	32.60	235.....	20.60	32.44
63.....	20.51	32.31			
93.....	20.33	32.02			

<sup>a</sup> 0.7925 N HCl is the acidity caused by 5 ml of 38.8 per cent HCl in 80 ml of solution.

TABLE 6.—Rate of Decrease of Clerget Divisor at Various Temperatures

[Caused by 0.7925 N HCl—Summary of Table 5]

Temperature	Decrease per minute
°C	°S
80	0.167
70	.056
60	.0132
50	.0034

The table shows in a concise form the great advantage of performing the Clerget analysis at temperatures lower than 70°. Not only can the time be regulated with greater precision when the reaction is slower, but the decomposition resulting from an error in timing is less than one-fourth as great at 60° and but one-sixteenth as great at 50°. In some of our preliminary experiments on the determination of the constant we varied the time of heating at 60° between 8 and 11 minutes and obtained practically the same value. Thus either the decomposition in its earlier stages is too small to be detected with sufficient precision, or there is a sort of induction period during which the invert sugar is more resistant to the action of the acid. The figures given by Herzfeld apparently show this same phenomenon. In certain modifications of Herzfeld's method adopted later the time was frequently extended to 10 minutes. According to curve No. 3, there are 5 minutes during which the decomposition is occurring. The invert polarization may, under these conditions, be nearly 0°.3 too low.

### III. APPARATUS AND MATERIALS

#### 1. SACCHARIMETERS AND TEMPERATURE CONTROL

The saccharimeters used and the many precautions taken to utilize their full sensibility have been described previously in a publication by Bates and Jackson.<sup>19</sup> In brief, the instruments used were the most recent Fric saccharimeter equipped with Bates's device for adjusting the sensibility, a Julius Peters saccharimeter, and, to a small extent, a Schmidt and Haensch saccharimeter. The Bates-Fric instrument was the main reliance. On the adjusting screws of these instruments were fitted long lever arms to provide for slow movement in making the settings. Bates and Jackson found that under the most favorable condi-

<sup>19</sup> B. S. Bulletin, 13, p. 101; 1916 (Scientific Paper No. 268).

tions the precision with which a measurement could be made with a saccharimeter approached very closely that attainable with a very sensitive circular-scale polarimeter illuminated with monochromatic light. The mean of their measurements is probably accurate to  $0^{\circ}.01$  S. Subsequently Jackson<sup>20</sup> found that measurements on the rotation of dextrose, in spite of a slightly heterochromatic field, were capable of nearly as high precision. Consequently, so far as the optical measurements were concerned, it was to be expected that invert-sugar solutions could be read to  $0^{\circ}.01$  S.

In order to attain this precision of measurement it is evidently necessary to control temperature with much greater care than in the case of sucrose or dextrose. This fact becomes obvious by referring to formula (3) on page 128. The denominator contains the correction term  $\frac{t}{2}$ . Hence a measurement valid to  $0^{\circ}.01$  S must be controlled to  $0^{\circ}.02$  C. The following precautions were taken to secure this control. As in the work by Bates and Jackson, the measurements were made in a thermostatted dark room. The saccharimeters were completely inclosed in a small wooden air bath equipped with an electrically operated thermostat. These thermostats secured a regulation of temperature somewhat closer than  $0^{\circ}.1$  C. In the present work a stream of water was pumped by a small centrifugal pump through the water jackets of the polariscope tubes. The water was taken from a thermostat which was constant to  $0^{\circ}.01$  C and led through vacuum jacketed glass ducts to the polariscope tubes. These ducts were surrounded by 3 inches of powdered cork. The polariscope tubes, which were of the ordinary inversion type, had the defect that the thermometer was immersed in a portion of the liquid which was not completely surrounded by the circulating water. Consequently, a change in the temperature of the environment influenced the thermometer more than it did the portion of the liquid through which the light passed. To remedy this defect a supplementary water jacket was constructed which was fed by the water which emerged from the jacket of the tube. This extra jacket fitted closely the jacket of the polariscope tube and completely surrounded all the exposed portions of the tubulature and the lower portion of the thermometer, leaving only a small hole through which the thermometer passed. The thermometer was read at

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<sup>20</sup> B. S. Bulletin, 13, p. 633; 1916 (Scientific Paper No. 293).



frequent intervals during the observations and showed that the control was all that could be desired.

The volumetric flasks were the same that were used in the researches on sucrose and on dextrose. They were recalibrated and were found to have retained their volume within about 0.002 ml.

Thermometers and weights were tested by the respective divisions of this Bureau by comparison with the official standards.

## 2. QUARTZ CONTROL PLATES

The negatively rotating quartz plates were part of a series of primary standards of this Bureau. They were of optically homogeneous quartz and of excellent quality. Their rotation had been measured by the Physikalisch-Technische Reichsanstalt in terms of wave length 5892.5 Å and by this Bureau in terms of wave lengths 5892.5 Å and 5461 Å. To convert these values into sugar degrees, the former was divided by the factor 34.620 and the latter by 40.690 in accordance with the measurements made by Bates and Jackson. These three independent sets of calibrations were in excellent agreement. The careful study which these plates have received will be described in a future publication of this Bureau. By using these conversion factors the negative side of the saccharimeter scale is calibrated in terms of the rotation of pure sucrose. All measurements on invert sugar were made at such concentrations that they were in close proximity to the rotations of the control plates. In this way the errors of graduation of the saccharimeter scales were eliminated.

## 3. MATERIALS

(a) SUCROSE.—The sucrose was prepared by crystalizing from aqueous solution in the manner described by Bates and Jackson. In brief, a 50 per cent solution was filtered, boiled in a vacuum, at a temperature of 35 to 40° C to 81° Brix and allowed to crystallize slowly during continuous agitation in a crystallizer. The crystals were separated in a centrifugal machine and washed with alcohol. They were shown to have the same high purity as the material prepared in the earlier work.<sup>21</sup>

(b) INVERT SUGAR.—A quantity of invert sugar was prepared by inverting sucrose at 55° C with invertase extracted from bottom yeast. At the completion of the inversion the invertase was destroyed by heating to 70 or 80° C for about an hour.

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<sup>21</sup> Bates and Jackson, B. S. Bulletin, 13, p. 67; 1916 (Scientific Paper No. 268).

(c) **HYDROCHLORIC ACID.**—As will appear subsequently, the value of the Clerget divisor is a function of the acidity of the solution. It is, therefore, necessary to use hydrochloric acid solutions of definite concentration or at least to know definitely the concentrations of the solutions used. In the literature on this subject authors have frequently stated that they used 5 cc of concentrated HCl to accomplish the inversion. Unfortunately, the chemically pure acid of commerce has apparently a very variable concentration in spite of a uniform designation of specific gravity on the label. For example, three lots of acid, labeled specific gravity 1.19, corresponding to about 38.8 per cent, were found to contain 34.6, 35.2, and 31.7 per cents, respectively.

Herzfeld in his research on the modified Clerget method prescribed 38 per cent acid. The Association of Official Agricultural Chemists specifies 38.8 per cent. We have found it more accurate and more convenient to dilute the concentrated acid to exactly half the concentration specified by the association. The obvious advantages are that the variations in the commercial acid can be readily corrected; that the dilute solution does not vary in strength by loss of acid, since the diluted acid has approximately the concentration of a constant boiling mixture; that the discomfort of pipetting a fuming acid is avoided; and that the danger of destruction of sugar by contact with 12 N acid before complete mixing is much reduced. It is, then, merely necessary to invert with 10 ml of the half-strength acid instead of 5 ml of concentrated acid. For convenient reference the data concerning the strengths and density of the HCl solutions, to which we shall refer, are collected in Table 7. The densities were computed from the data in Landolt and Börnstein's tables.

Through a slight error in computation the acid used in our final series of measurements was slightly more than half as concentrated as that of the association. Instead of 6.34 N, it was actually 6.399 N, as determined by density measurements. This value was checked by a silver-chloride precipitation which gave 6.403 and by titration against alkali standardized by  $\text{H}_2\text{SO}_4$ , the strength of which was determined by a barium-sulphate precipitation. This gave the value 6.399. Since we have determined precisely the variation of the constant with variation of acidity, this slight difference in strength of acid causes no difficulty. The strengths of the two more dilute solutions were determined by titration.

TABLE 7.—Data on Concentrations of Hydrochloric Acid Solutions Used in Present Investigation

	Concentration of HCl, by weight	$d_{40}^{20}$	Brix	Normality	Weight, HCl per milliliter	Weight, NaCl after neutralization, per milliliter HCl
	Per cent				g	g
A. O. A. C. ....	38.8	1.1918	42.96	12.68	0.4623	0.7413
Herzfeld .....	38.0	1.1882	42.28	12.39	.4517	.7243
J. and G. ....	21.14	1.1038	25.05	6.399	.2333	.3741
A. O. A. C. ....	20.97	1.1029	24.85	6.340	.2312	.3706
2 .....						
J. and G. ....				3.202	.1167	.1872
J. and G. ....				0.804	.0293	.0470

#### IV. DETERMINATION OF THE CLERGET DIVISOR IN THE PRESENCE OF 0.634 N HCl

##### 1. FINAL SERIES OF MEASUREMENTS

By employing the data obtained in the foregoing experiments it is possible to determine the time required to complete the reaction and have the assurance that it is complete within the limits of accuracy of observation, and that the subsequent decomposition of the invert sugar is avoided. It has been shown on page 139 that in Herzfeld's method of inversion the temperatures prescribed are so high that the reaction completes itself almost by the time the solution reaches the temperature of the bath and that thereafter the time is so prolonged that serious decomposition ensues. Furthermore, it is not feasible for accurate measurement, to shorten the time to the  $5\frac{1}{2}$  minutes which curve No. 3 shows to be sufficient, because then the latitude for differences in individual procedure is so small that the danger of over or under timing is very great. Consequently, Herzfeld's procedure, in spite of its almost universal prevalence, is deemed unsuitable.

Before undertaking our final series of measurements, a considerable number of preliminary determinations were made which are not reported here, but which led to approximately the same numerical value as the final series. As a result of these preliminary trials we determined that the reproducibility of polariscopic observation by different observers is satisfactory; that the type of saccharimeter is without influence on the result; that the temperature coefficient is valid even to  $30^{\circ}\text{C}$ ; and that the concentration correction now in use is reliable.

Having learned that the final value was independent of the type of saccharimeter, we determined all the values obtained in the final series on the Bates-Fric instrument because of its greater precision.

Three variations of procedure were used, each of which has been shown on pages 138 to 141 to yield a completely inverted solution with no subsequent decomposition.

In the first five experiments the inversion was carried out at a constant temperature of 60°. We have shown on page 138 that the velocity constant ( $k$ ) at 60° is 0.4606. Consequently, the time required for 99.99 per cent inversion is given by the formula

$$t = \frac{1}{0.4606} \log \frac{100.00}{100.00 - 99.99} = \frac{4.0}{0.4606} = 8.7 \text{ minutes.} \quad (12)$$

In a number of preliminary experiments we obtained identical results when the time of heating was varied between 8 and 11 minutes.

The procedure was as follows: A stock of sugar, previously dried in a vacuum over lime, was kept in a desiccator. The pure, dry sugar was transferred through a funnel to a weighed volumetric flask and weighed, after which the residual moisture content, which never exceeded 0.005 per cent and was usually much less, was determined by heating in a vacuum in the manner described by Bates and Jackson.<sup>22</sup> The sugar was dissolved in a quantity of distilled water sufficient to make the solution occupy a volume of 70 ml. The containing flask was suspended in the thermostat set at 60° C and allowed to assume the temperature of the bath. The acid, similarly suspended in a test tube, contained the same weight of anhydrous HCl in 10 ml at 20° C as would 5 ml of 38.8 per cent acid, namely, 2.312 g. A 10 ml pipette was calibrated to deliver at 60° C a volume of acid which, when cooled to 20°, occupied 10 ml. When both solutions had attained the temperature, the acid was pipetted into the sugar solution, shaken to mix, and the inversion carried out. In 9½ minutes the solution was removed from the bath, rapidly cooled, and placed in a thermostat maintaining its temperature accurately at 20°. After equalization of temperature, it was made to volume, mixed, and poured into the polariscope tubes. Each solution was observed in two polariscope tubes in order to detect or diminish extraneous

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<sup>22</sup> B. S. Bulletin, 13, p. 91, 1916 (Scientific Paper No. 268).

effects, such as pressure on the cover glasses or errors in observation of temperature. The tubes were placed in the air bath surrounding the saccharimeters and their jackets connected with the ducts through which the water from the water thermostat circulated.

The readings were always made by both of the authors independently. Each observer read successively the control plate, tube No. 1, tube No. 2, control plate, tube No. 1, tube No. 2, and control plate. Each time the plate or tube was read from 6 to 10 settings of the end point were made. A complete record was kept of the temperatures of solutions, quartz wedges, and the air in the air bath.

In Experiment No. 6 the inversion was accomplished by adding 10 ml of the acid to 70 ml of solution, both at room temperature. The acidified solution was then placed in the bath at 60°, agitated for 3 minutes and allowed to remain in the bath until the reaction was complete. Curve No. 2, Fig. 1, shows that, under these conditions, inversion is complete in 9 minutes. The surprising result, apparently, is that the solution, heated from the temperature of the room, inverts in nearly the same total time as that which was initially at 60°.

In experiments Nos. 7 and 8 the procedure suggested by Walker was employed. The sugar solution occupying a volume of 70 ml was heated to 65° C, 10 ml of HCl ( $d_{40}^{20} 1.1029$ ) was added, the flask set on the laboratory table and allowed to cool during the inversion. After the lapse of 15 minutes, the flask was placed in the thermostat and made to volume at 20° C.

The results of these measurements are summarized in Table 8, columns 5 and 6, and show the mean of the readings taken by the individual observers. If each of these columns is averaged, it will be found that they are in agreement within 0.008 S. The third decimal figure is, of course, without significance, except in so far as it assists in determining the value of the second figure.

**TABLE 8.**—Summary of Measurements on the Rotation of Invert Sugar in the Presence of 0.634 N Hydrochloric Acid

Experiment No.	Weight sugar (air, brass weights)	Volume of solution	Concentration of sucrose in solution	Observed rotation		Temperature of observation	Rotation corrected for temperature and concentration
				Observer J	Observer G		
	g	ml	g/100 ml	°S	°S	°C	°S
1.....	25.175	130.004	19.365	−25.05 <sub>1</sub>	−25.05 <sub>9</sub>	20.14	−43.27
2.....	25.382	130.016	19.523	−25.31 <sub>1</sub>	−25.29 <sub>2</sub>	20.05	−43.28
3.....	25.428	130.014	19.557	−25.33 <sub>6</sub>	−25.31 <sub>8</sub>	20.11	−43.29
4.....	30.209	130.016	23.234	−30.26 <sub>7</sub>	−30.22 <sub>0</sub>	20.07	−43.21
5.....	20.742	100.185	20.704	−26.86 <sub>5</sub>	−26.87 <sub>3</sub>	20.09	−43.27
6.....	15.902	100.183	15.873	−20.39 <sub>3</sub>	−20.41 <sub>5</sub>	20.01	−43.24
7.....	15.892	100.185	15.863	−20.41 <sub>5</sub>	−20.39 <sub>0</sub>	20.07	−43.29
8.....	15.784	100.000	15.784	−20.26 <sub>6</sub>	−20.27 <sub>8</sub>	20.11	−43.26
Average.....							−43.26
Correction from 0.640 N to 0.634 N acid.....							+ .01
Corrected value.....							−43.25

The value of the constant was computed by the formula

$$C = \left( \text{observed rotation} \frac{26.000}{\text{g of sucrose}/100\text{ml}} \right) - \frac{t}{2} - \text{concentration correction.} \quad (13)$$

For the observed rotation the mean of columns 5 and 6 was taken. As stated above, we have found that the values of the concentration corrections already in use were reliable. The correction was found from the equation

$$\text{Concentration correction} = (m - 13) 0.0676, \quad (14)$$

where  $m$  is the number of grams of sucrose in 100 ml. The rotations, corrected for temperature and concentration, are recorded in column 8. The values represent twice the rotation of 13 grams of sucrose in 100 ml of solution, inverted by 5 ml of 38.8 per cent HCl, observed in a 200 mm polariscope tube at 20° C and augmented by the temperature correction  $\frac{20^{\circ}.00.}{2}$ . They are thus comparable

in every way with the previously determined values of Herzfeld. The average of the eight determinations is −43.25. This value is valid only in the presence of 0.634 N HCl, the acidity produced in 100 ml by 5 ml of 38.8 per cent acid.

In recapitulation we have selected in each of the three methods of inversion the proper length of time for a completed reaction and have avoided the decomposition which would ensue if the time were too prolonged or the temperature too high. Herzfeld's value of this constant was −42.66. We have shown that by his pro-



cedure a decomposition of invert sugar must occur, and the conclusion is drawn that the value of the constant proposed by him is too low by  $0^{\circ}.60$  S. With reference to the quantity actually measured—namely,  $-33.25$ , the rotation of invert sugar at  $20^{\circ}$ —this error is nearly 2 per cent.

## 2. DISCUSSION

We have endeavored to assign limits to the possible error of the value given in Table 8. The "probable error"  $r$  of the mean, computed from the least square equation,

$$r = 0.6745 \sqrt{\frac{\sum v^2}{n(n-1)}},$$

in which  $v$  is the deviation of each measurement from the mean and  $n$  the number of measurements, proves to be  $0^{\circ}.007$  S. Besides the "probable error" there still remains the possibility of constant errors affecting the result. These may arise either during the preparation of the solutions or from the polariscopic observations. We have discussed the preparation of the solutions at considerable length and have tried to show that each detail in the procedure was carried out with a far greater refinement than the polariscopic observations are capable of. Such error, then, as may exist in the final result is probably to be ascribed to the reading of the saccharimeter. Since we have made no measurements of the rotation of invert sugar with monochromatic light, we have no data by which to compare its rotary dispersion with that of quartz. However, we were unable to detect the presence of any uncompensated color in the field of the saccharimeter. The essential requirement is that the value be reproducible. At present we may cite the essential agreement between the two authors, the mean variation being but  $0^{\circ}.008$  S. It seems probable, then, that the value  $-43.25$  is accurate to  $0^{\circ}.01$  or  $0^{\circ}.02$ . It is hoped that other investigators will make measurements of this important constant.

It would be of much interest if, in the light of our experiments, we could account for the low result of Herzfeld's measurements. Unfortunately, many essential details are omitted in the original paper, and the following discussion is consequently somewhat conjectural.

Herzfeld's communication was published in 1888. He did not state upon what basis his saccharimeter scales were standardized, nor did he state whether the unit of volume he used was the true

cubic centimeter or the Mohr unit, prevalent at that time. He made all his measurements at 20° C. If he used 13.024 g of sucrose in 100 true cubic centimeters and based his measurements on the Ventzke scale standardized by the conversion factor 34.69, his concentration of sugar should have given a value 0.2 per cent higher than ours; but, on the other hand, the scale would have reduced this by 0.2 per cent in comparison with ours, which was based upon the conversion factor 34.620. If he used 13.024 g in 100 Mohr cubic centimeters the concentrations were approximately the same, but the difference in the basis of standardization of the scale would then have reduced his readings about 0.2 per cent lower than ours. In neither case is the possible difference in standardization sufficient to account for the 2.0 per cent variance in the final result.

It is probable that Herzfeld's light source was not filtered through a bichromate solution, since the latter did not come into general use until about 1900; so it was thought possible that difference in saccharimeter illumination might have affected the observed rotations. We, therefore, ascertained by direct measurement the magnitude of this effect. A solution, which gave a corrected polarization of  $-42.36$  with the bichromate filter in the optical path, gave a value of  $-42.49$  with the filter removed. If, therefore, Herzfeld's measurements were made with unfiltered light, his constant would have become still lower upon using the bichromate filter.

Impurities in the sugar can not conceivably have been present in sufficient quantity to have affected the results. Herzfeld used chemically pure sucrose from two different sources, and the careful manner in which we have prepared ours we have already described.

There remains to be considered the destructive effect of the acid during, and subsequent to, the inversion. Curve No. 3, page 140, shows the progress of the reaction under the conditions prescribed by the Association of Official Agricultural Chemists. The conditions are slightly more favorable to destruction of invert sugar than in Herzfeld's original experiments. He used 38.0 per cent HCl, whereas the association prescribes 38.8 per cent. In every experiment but one Herzfeld diluted this acid with a greater volume of solution than in the association's method. It is, therefore, to be expected that in his experiments the invert sugar, would suffer slightly less decomposition than is shown by curve No. 3. However, by applying the data represented by this curve we can

obtain his maximum probable decomposition. An invert-sugar solution containing 5 ml of 38.8 per cent HCl is decomposed by the acid at 70° C, an amount each minute which corresponds to a diminution of the constant of 0.°056 S. If inversion is complete in 5½ minutes from the starting of the reaction, and the solution remains 2 minutes longer, making the 7½ minute period which Herzfeld prescribed, there results a decrease of 0.°11 S in the value of the constant. Conceivably the destruction may occur before the inversion is complete to an extent which can not be readily estimated. However, even if we assume that decomposition occurs during the 3 or 4 minutes when the solution is at 70°, it is difficult to understand how the decomposition can effect a diminution of the rotation greater than 0.°15 or 0.°20 S. If this amount were deducted from our value, we would obtain as the negative constituent of the Clerget divisor from -43.06 to -43.11. Steuerwald,<sup>23</sup> in a careful revision of Herzfeld's experiment, obtained the quantity -43.05.

Our measurements on the rate of decomposition apply specifically to the carefully controlled conditions under which they were conducted. It is difficult to say how much the rate is affected when these conditions no longer obtain. Since in Herzfeld's procedure the specified time of heating is on a descending portion of the curve (see Fig. 1), the particular value obtained would depend upon the minute details of individual procedure. This fact precludes the possibility of reproducibility by different individuals. In illustration of this we may cite four out of the great number of determinations in which Herzfeld's modification has been employed. Herzfeld himself obtained the value -42.66; Walker, -42.78<sup>24</sup>; Tolman, -42.88;<sup>25</sup> and Steuerwald, -43.05. Here is a maximum difference of 0°.4 S obtained by careful investigators using the same method. This condition is faulty enough, but is still further aggravated in some instances by a further extension of the time. For instance, the Association of Official Agricultural Chemists,<sup>26</sup> ostensibly adopting Herzfeld's procedure, extended the time to a total of 10 minutes, although the strength of the catalyzing acid was slightly increased. It is perhaps, due to these unfortunate conditions in the basic research on the subject and the modifications adopted subsequently that the Clerget method has acquired the reputation of being an ap-

<sup>23</sup> Int. Sugar. Journ., 16, p. 82; 1914.<sup>24</sup> Sugar, 17, No. 2, p. 47; 1915.<sup>25</sup> Bull. Bur. Chem., 73, p. 73; 1903.<sup>26</sup> Bull. Bur. Chem., 107, p. 41.

proximation.<sup>27</sup> The authors of the present paper contend that if the inversion is carried out under such conditions that the end of the reaction is situated on a broad and flat portion of the curve, relatively large differences in individual procedure can be tolerated without a diminution of accuracy. Under such conditions, the accuracy of the Clerget divisor itself is limited only by the precision with which polariscopic observations can be made. The remaining sources of error, due to the presence of impurities, will be discussed in detail in the following sections of this paper.

## V. INFLUENCE OF THE PRESENCE OF IMPURITIES ON THE CLERGET METHOD

Inasmuch as the prime purpose of the Clerget method is the determination of sucrose in the presence of impurities, it is necessary to study the behavior during the course of the analysis of such impurities as commonly occur in sugar products. In the sugar industry these impurities may be divided fairly sharply into four classes: (1) Invert sugar, which occurs in cane products and but rarely in beet products; (2) the nonsugars of the cane; (3) the nonsugars of the beet; (4) raffinose, which occurs in beet products.

The modifications which the Clerget method requires if invert sugar is present we shall discuss in the following pages of this section. The presence of nonsugars of the cane does not necessitate any modification in the method unless they change their optical activity upon acidification. If they do change upon acidification, they may be analyzed by a method (II, on p. 184) which avoids polarization of the invert solution in the presence of acid. The nonsugars of the beet frequently contain optically active substances whose activity is influenced by the presence of acid. We suggest below, under the third part of Section VI, the modification of the method which overcomes this inequality of rotation and describe in method III on page 186 the procedure to be followed if these substances are present. The presence of raffinose does not require a modification in this method, but does require a different method of calculation. This is discussed in the fourth part of Section VII.

### 1. EFFECT OF REAGENTS ON THE ROTATION OF INVERT SUGAR

Invert sugar is practically always present in all cane products except refined sugar. In molasses it may be as high as 25 per cent or more of the total substance. Inasmuch as our require-

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<sup>27</sup> Browne, *Handbook of Sugar Analysis*, p. 278.

ment for an exact Clerget method is that the rotation of all substances except sucrose shall remain unchanged during the analysis, it is necessary to see how far this condition obtains and what modifications are needed to secure constancy.

(a) HYDROCHLORIC ACID.—Since hydrochloric acid is the inverting agent used for the analytical method, its effect on the rotation of invert sugar was first studied. This effect was measured quantitatively in two ways, each of which possesses a special significance for our present purpose. One method, which will be described on page 165, was to start with pure sucrose and invert with acids of varying concentration. The other method was to add varying amounts of HCl to the pure invert sugar.

In the latter method, the invert sugar was prepared by inverting refined sugar by invertase as described on page 145. The solutions were made up by pipetting 13 to 20 ml of invert sugar solutions into weighed 25 ml picnometers, which were then reweighed. The HCl was added from a graduated 2 ml pipette and the solution made to volume. In each set of determinations three solutions were made up, one of which served as a control. For the control solution a quantity of acid was added, such that its concentration was the same as that in the series of determinations described on pages 147 to 151. Its rotation was then multiplied by such a factor that the product would be  $-43.25$ , diminished by one-half the temperature of observation. This procedure is tantamount to a standardization of the solution by means of its rotation, on the basis of the value  $-43.25$ . Inasmuch as the rotations of the other two solutions were multiplied by the same factor, a series of comparative results were obtained which were based on the value  $-43.25$  as a standard and which were free from any uncertainty about incomplete inversion or decomposition. The factor was found by the equation

$$\frac{\text{Rotation} \times \text{volume of picnometer}}{\text{Weight of solution}} \times \text{Factor} = -43.25 + \frac{t}{2} \quad (15)$$

The slight differences in volume delivered by the pipette and in the picnometers were eliminated by the calculation. The values obtained are summarized in Table 9 and plotted in Fig. 2. They show that within wide limits the relationship is linear, even when the amount of acid varies between 0 and 40 ml of 6.4 N HCl per 100 ml of solution. This is contrary to the findings of Tolman.<sup>28</sup> The same linear relation was found in the case of

<sup>28</sup> Bull. Bur. Chem., 73, p. 70; 1903.

other reagents. The significance of the results will be seen when compared with those obtained by the other method which will be discussed in connection with this on page 166. Suffice it to say here that the effect of hydrochloric acid on the rotation of invert sugar may be stated in terms of the negative constituent of the Clerget divisor by the equation

$$R = -42.00 - 0.125 C, \quad (16)$$

in which  $C$  represents the number of ml of 6.34 N HCl in 100 ml of solution, or

$$R = -42.00 - 0.5407 m = -42.00 - 1.972N \quad (17)$$

in which  $m$  represents the number of grams of anhydrous HCl in 100 ml of the solution polarized, and  $N$  the normality of the solution with respect to the acid.

In other words, the rotation of invert sugar is in aqueous solution  $-42.00$  and in acid solution  $-43.25$ . Here at once we have a very serious source of error, inasmuch as in the analysis the change of rotation of invert sugar which is already present in the original sample would, by the Clerget formula, be reported as sucrose. This fact has long been known to sugar analysts, but thus far no effective remedy has been suggested. We have measured it quantitatively with this end in view.

It would be desirable to find some reagent to add to the direct polarization which would have the same effect on the invert sugar as the hydrochloric acid in the invert polarization. The only reagent at present added to the direct polarization after clarification with basic lead acetate is acetic acid to a slight acidity. It is added for the purpose of decomposing a lead-levulose compound.

(b) ACETIC ACID.—The authors shared the general impression that the effect caused by hydrochloric acid on invert sugar was due to its acidity. We therefore sought to produce the same effect on invert sugar in the direct polarization by adding acetic acid in greater excess than that required to decompose the lead-levulose compound.

Weber and McPherson <sup>29</sup> attempted to replace hydrochloric acid by acetic acid in the Clerget analysis. They found a comparatively low value for the negative rotation in acetic solution. Aside from this, no measurement of the effect of acetic acid on invert sugar has apparently been made.

In making our measurements we took as the control solution either one containing hydrochloric acid of strength corresponding

<sup>29</sup> J. Amer. Chem. Soc., 17, p. 321; 1895.

to  $-43.25$  or an aqueous solution to which we assigned the value  $-42.00$  found from the HCl curve. As appears from Table 9 and Fig. 2, an increase in the quantity of acetic acid causes a diminution of the negative rotation of invert sugar. Hence in the Clerget analysis it would but serve to aggravate the effect which it was intended to correct.

(c) PHOSPHORIC ACID.—Under the supposition that acidity was a necessary qualification for increasing the negative rotation of invert sugar, the effect of phosphoric acid was measured. As is shown in Table 9 and Fig. 2, it produces a marked elevation. It is, however, quite unsuitable for the purpose at hand, because it is strong enough to cause a very considerable inversion of sucrose during the length of time necessary for observing the direct polarization. Consequently, we turned our attention to measurements of the effects of neutral salts on the rotation of invert sugar.

TABLE 9.—Showing the Effect of the Presence of Various Reagents on the Rotation of Invert Sugar

Weight per 100 ml, in grams	Normality of reagent	Rotation $\times 2$ of 13 g inverted sucrose $\frac{t}{2}$	Calculated rotation	Weight per 100 ml, in grams	Normality of reagent	Rotation $\times 2$ of 13 g inverted sucrose $\frac{t}{2}$	Calculated rotation
HCl				NaCl			
$R = -42.00 - 0.5407 m$				$R = -42.00 - 0.540 m$			
		$^{\circ}S$	$^{\circ}S$			$^{\circ}S$	$^{\circ}S$
0.4649	0.1274	-42.19	-42.25	.4703	0.0804	-42.24	-42.25
.5343	.1465	-42.32	-42.29	1.870	.3199	-43.01	-43.01
.9262	.2539	-42.47	-42.50	2.945	.5038	-43.60	-43.59
1.177	.3230	-42.56	-42.64	3.741	.6399	-44.02	-44.02
2.312	.634	.....	-(43.25)	CaCl <sub>2</sub>			
3.801	1.045	-44.09	-44.06	$R = -42.00 - 0.710 m$			
4.742	1.301	-44.55	-44.56	0.8165	0.1471	-42.54	-42.58
9.322	2.556	-46.97	-47.04	1.633	.2942	-43.22	-43.16
K <sub>2</sub> C <sub>2</sub> O <sub>4</sub>				2.467	.4445	-43.75	-43.75
$R = -42.00 - 0.510 m$				2.509	.4521	-43.78	-43.78
0.6405	0.0771	-42.18	-42.33	3.363	.6060	-44.68	-44.39
.6681	.0804	-42.43	-42.34	7.501	1.351	-47.52	-47.33
1.352	.1628	-42.73	-42.69	HC <sub>2</sub> H <sub>3</sub> O <sub>2</sub>			
2.712	.3263	-43.30	-43.38	$R = -42.00 + 0.0823 m$			
3.375	.4061	-43.76	-43.72	4.2048	0.7008	-41.68	.....
3.964	.4770	-44.10	-44.02	20.676	3.446	-40.25	.....
12.87	1.549	-47.73	-47.81	40.968	6.828	-38.47	.....
NH <sub>4</sub> Cl				H <sub>3</sub> PO <sub>4</sub>			
$R = -42.00 - 0.563 m$				$R = -42.00 - 0.0776 m$			
1.899	0.355	-43.07	-43.07	0.4510	0.1380	-42.16	.....
3.392	.634	-43.91	-43.91	2.157	.6602	-42.53	.....
3.683	.688	-44.09	-44.07	5.505	1.684	-43.25	.....

## OTHER REAGENTS

Reagent	Weight per 100 ml	Normality of reagent	Rotation $\times$ 2 of 13 g inverted sucrose $\frac{t}{2}$	Change in rotation per gram
			$^{\circ}\text{S}$	$^{\circ}\text{S}$
$x\text{Pb}(\text{C}_2\text{H}_3\text{O}_2)_2 \cdot y\text{PbO}$ .....	2.570	0.187	-38.32	+1.43
$\text{Pb}(\text{C}_2\text{H}_3\text{O}_2)_2$ .....	3.031	.186	-42.06	— .020
$\text{NH}_4\text{NO}_3$ .....	2.683	.335	-43.07	— .399
$\text{KCl}$ .....	11.83	1.59	-47.75	— .486
$\text{Na}_2\text{HPO}_4 \cdot 12 \text{H}_2\text{O}$ .....	12.26	1.03	-43.97	— .161
$\text{NaC}_2\text{H}_3\text{O}_2 \cdot 3\text{H}_2\text{O}$ .....	12.85	.944	-44.43	— .189

(d) SODIUM CHLORIDE.—A series of comparative determinations of the effect of salt solutions on the rotation was made in the manner described in the case of hydrochloric acid. For the control solution either the aqueous invert solution (rotation  $-42.00$ ) or the  $\text{HCl}$  solution (rotation  $-43.25$ ) was used. Preliminary experiments showed the marked effect in the desired direction which it exerts. Inasmuch as this effect was measured much more precisely in the series of absolute determinations, described on pages 169 to 171, the values plotted and the equation expressing it are taken from the later measurements. The rotation in terms of the negative constituent of the Clerget divisor is expressed by the equation

$$R = -42.00 - 0.540 \, m = -42.00 - 3.157 \, N \quad (18)$$

in which  $m$  is the number of grams of sodium chloride in 100 ml of the solution polarized, and  $N$  the normality of the solution with respect to salt. By comparison of this formula with that expressing the effect of hydrochloric acid, we find that 1 g of salt produces almost identically the same effect as 1 g of hydrochloric acid. Contrary, therefore, to the general impression, it is not because of its acidity that hydrochloric acid exerts its influence. This effect is but a single instance of the general truth that all dissolved substances to a greater or less degree affect the rotation of all optically active substances. As far as can be stated now, the effect of inorganic substances on the rotation of invert sugar is a complicated function of both ions and can in no instance be directly ascribed to mere acidity or neutrality.

Inasmuch as equal weights of salt and hydrochloric acid exert the same influence on invert sugar, it is obvious that upon neutralization of hydrochloric acid after inversion the weight of salt will be about 1.6 times as great as the weight of the acid. As we



shall show later, the rotation of the neutralized invert sugar solution does show an elevation in negative rotation about 1.6 times as great as that of the acid solution.

Sodium chloride is quite without inverting action on sucrose and has the added advantage that, under suitable conditions, it precipitates the excess lead from the clarified solution. The only conceivable disadvantage that it might have is that the solubility of lead chloride is a relatively large function of the temperature. An analyst might in haste make his solution to volume and filter before it had reached the temperature of polarization. If it cooled subsequently, lead chloride would precipitate in the polariscope tube.

(e) POTASSIUM OXALATE.—A saturated solution of potassium oxalate was prepared by agitating the crystals in distilled water at the temperature of the laboratory. The solution was filtered and analyzed by titration against potassium permanganate standardized by pure sodium oxalate. The solution contained 332.6 g  $K_2C_2O_4$  per liter, corresponding to a normality of 4.002.

Its influence on the rotation of invert sugar was measured in the manner already described. The values obtained are given in Table 9 and plotted in Fig. 2. The effect on the negative constituent of the Clerget divisor is expressed by the equation

$$R = -42.00 - 0.510 m = -42.00 - 4.239 N, \quad (19)$$

in which  $m$  represents the number of grams of anhydrous potassium oxalate in 100 ml of the solution polarized and  $N$  the normality of the solution with respect to  $K_2C_2O_4$ . It is thus evident that potassium oxalate may be used effectively to increase the rotation of invert sugar.

(f) CALCIUM CHLORIDE.—Calcium chloride may conceivably be used advantageously when it is necessary to add a highly concentrated solution. The solution employed was analyzed by a silver chloride precipitation and contained 0.2055 g per milliliter, corresponding to a normality of 3.704. Its effect on the negative constituent of the Clerget divisor is expressed by the equation:

$$R = -42.00 - 0.710 m = -42.00 - 3.941 N, \quad (20)$$

in which  $N$  is the normality of the salt and  $m$  is grams of anhydrous calcium chloride in 100 ml of the solution polarized. The values obtained are given in Table 9 and plotted in Fig. 2. As

equation (20) shows, calcium chloride increases the rotation of invert sugar effectively.

(g) AMMONIUM CHLORIDE.—We shall describe below some experiments which show that ammonium chloride may be used

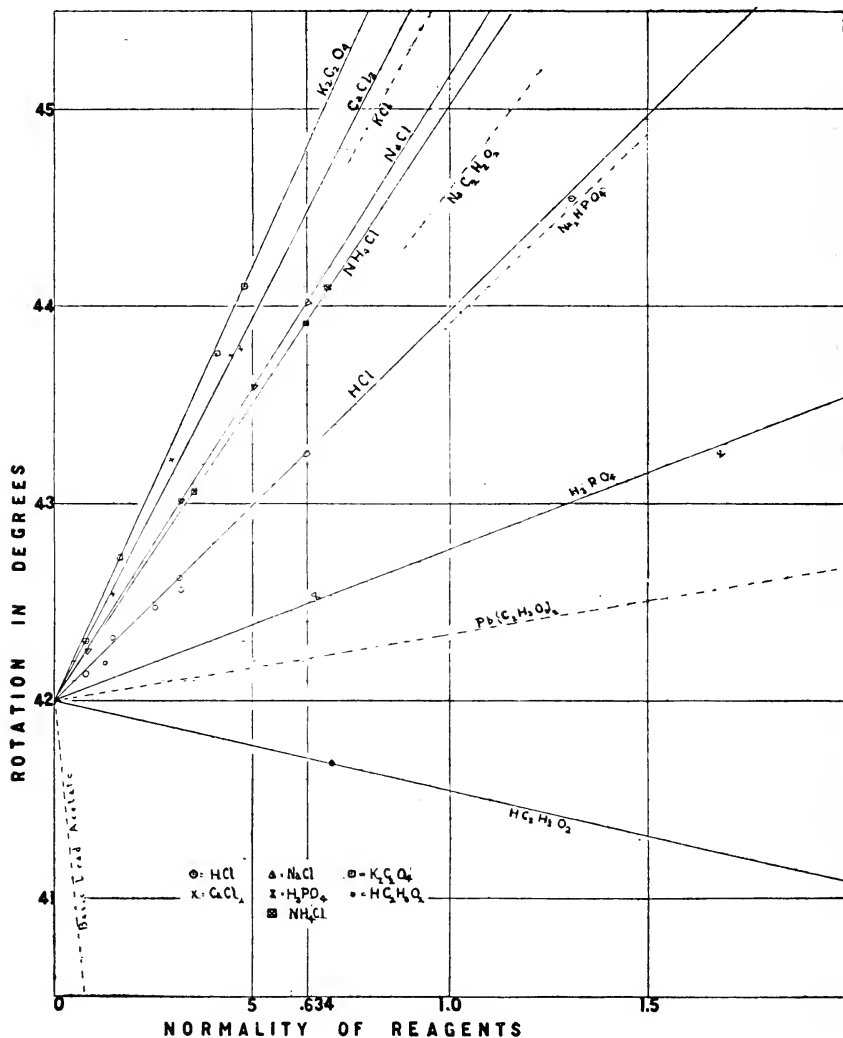


FIG. 2.—The influence of the presence of reagents on the rotation of invert sugar

Normality 0.634 is produced by 5 ml of concentrated hydrochloric acid in 100 ml of solution. The effect on the rotation caused by neutralizing the acid is seen by following the 0.634 ordinate to its intersection with the ammonium, sodium, potassium, or calcium chloride curves.

advantageously in certain modifications of the Clerget method. We have, therefore, determined its influence on the rotation of invert sugar. The three experiments in Table 9 show that the

influence is linear with the concentration of the salt. The relation is expressed by the equation:

$$R = -42.00 - 0.563 m \quad (21)$$

in which  $m$  represents the number of grams of ammonium chloride in 100 ml of the solution polarized.

(h) BASIC LEAD ACETATE.—A basic lead acetate solution was prepared by dissolving the solid crystals in water and diluting to specific gravity 1.25. It was analyzed by the oxalate method described by Jackson<sup>30</sup> and was found to contain 0.2072 g of  $\text{Pb}(\text{C}_2\text{H}_3\text{O}_2)_2$  and 0.1077 g of  $\text{PbO}$  per milliliter. The solution was, therefore, 0.966 N with respect to  $\text{PbO}$  and 1.273 N with respect to  $\text{Pb}(\text{C}_2\text{H}_3\text{O}_2)_2$ , or 2.296 N with respect to both constituents. To neutralize 1 ml of the basic solution, 0.966 ml of normal acetic acid was required.

The well-known effect of basic lead upon the rotation of invert sugar is illustrated by the measurement in Table 9. The effect was so great that the point could not be plotted on the scale on which Fig. 2 is plotted, but the direction of the point is shown by the dotted line.

If the solution containing basic lead is neutralized by adding the proper quantity of acetic acid, the rotation becomes but slightly greater than its value in pure aqueous solution.

(i) OTHER REAGENTS.—In our search for suitable reagents to produce the desired effect on invert sugar, we measured the influence of a number of other salts. These measurements are recorded in Table 9 and plotted in Fig. 2. Whenever the scale of the diagram would not accommodate the normality of the reagent added, the direction of the plotted point is indicated by a dotted line.

(j) CONCLUSION.—We have shown above that there is a large class of neutral salts which elevate the negative rotation of invert sugar in the same manner in which hydrochloric acid does. If, then, the mixture to be analyzed consists of invert sugar and sucrose, it is merely necessary to add to the solution which is to be polarized directly a quantity of the salt sufficient to raise the rotation of the invert sugar to the same value which it will have in the presence of hydrochloric acid in the invert polarization.

Inasmuch as the relation is a linear one up to relatively high concentration of the salts, the presence of a considerable quan-

<sup>30</sup> B. S. Bulletin, 11, p. 333; 1914 (Scientific Paper No. 232).

tity of inorganic material in the original sample, such as occurs in molasses, has no influence on the method, since obviously this will affect the invert sugar equally in both polarizations

If 5 ml of 38.8 per cent HCl or 10 ml of 6.34 N HCl is used in 100 ml for the invert polarization, the weights of salts to be added to the solution for the direct polarization are given in column 2 of Table 10. In column 3 is given the volume of a saturated solution containing the corresponding weights of sodium chloride or potassium oxalate. This volume can be conveniently measured from a 10 ml graduated pipette. Obviously the saturated solution may be diluted so that the required weight of salt is contained in any conveniently measured volume. In column 4 is given the concentration of a salt solution which, if added to a solution which is to be diluted one-tenth, will contain enough salt to supply this weight to 100 ml of the diluted solution. It is in this connection immaterial what the actual volumes taken are, whether they be 50 to 55 or 100 to 110 ml. In column 5 is given the weight of the salt to be added for each milliliter of normal acid when the inversion is brought about by acid of any given concentration. If, for example, 10 ml of 4 N HCl is added and made up to 100 ml for the invert polarization, this as equivalent to 40 ml of N HCl. The weight of sodium chloride to be added to the solution to be polarized directly is

$$40.0 \times 0.03651 = 1.460 \text{ g to 100 ml.}$$

TABLE 10.—Quantity of Reagent to be Added to the Solution for Direct Polarization in Order to Influence the Rotation of Invert Sugar by the Same Amount as Hydrochloric Acid

Salt	Weight of salt causing same change in rotation as 5 milliliters 38.8 per cent HCl	Volume of saturated solution containing same weight	Concentration of salt to be used for one-tenth dilution	Weight of salt required for each milliliter of normal HCl used in inverting
	g	ml	g/liter	g
NaCl.....	2.315	7.145	254.65	0.03651
K <sub>2</sub> C <sub>2</sub> O <sub>4</sub> .....	2.451	7.369	269.61	.03866
CaCl <sub>2</sub> .....	1.761	.....	193.71	.02777

## 2. EFFECT OF REAGENTS ON THE ROTATION OF SUCROSE

Having found a suitable group of reagents for producing the needed increase in the rotation of invert sugar, we next proceeded to measure the influence which these exert upon the rotation of sucrose. Both acetic and phosphoric acids were found to cause an inversion of sucrose to a degree that rendered them quite unsuitable for the purpose at hand. On the other hand, the three neutral salts—sodium chloride, potassium oxalate, and calcium chloride—answered the requirements satisfactorily. Their effects upon the rotation of sucrose were then carefully measured.

Solutions of chemically pure sucrose were prepared and the reagents added by pipetting the desired quantity from stock solutions the concentration of which had been carefully ascertained by previous analysis. Each sucrose-salt solution was then made to volume at 20° C and polarized. The rotations were observed at 20° with as much care and with as many settings of the instrument as in the measurements on the inverted solutions. The experiments described here were confined to the normal concentration <sup>31</sup> of sucrose.

The results of these measurements on normal sucrose solutions are collected in Table 11. As is evident, the rotation of sucrose is diminished in a linear relation with the quantity of salt added. Furthermore, if the concentration of the salt is measured with care, the rotation of sucrose in the presence of salt is capable of the same accuracy of measurement as the rotation of pure sucrose solutions. We can, therefore, use for a given salt concentration the corresponding rotation of sucrose with the same confidence that we use that of the normal weight of sucrose in pure aqueous solution.

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<sup>31</sup> A research by Bates and Jackson is now in progress on the variation of the specific rotation of sucrose with concentration for the purpose of establishing the complete sugar scale for any concentration or any desired normal weight. It was therefore deemed premature to investigate the effect of salts on sucrose in concentrations at which the pure substance was still in a state of uncertainty. We contented ourselves for the time being with a few experiments at varying concentrations to show that the Clerget method was sufficiently valid at other than normal concentrations.

TABLE 11.—Showing the Effect of the Presence of Various Reagents on the Rotation of Sucrose

Concentration of reagent, in grams per 100 milliliters	Rotation observed	Rotation calculated	Concentration of reagent, in grams per 100 milliliters	Rotation observed	Rotation calculated
NaCl			$R=100-0.265\ m$		
	°S	°S		°S	°S
0.311.....	99.95	99.92	0.665.....	99.83	99.84
.933.....	99.75	99.75	.998.....	99.78	99.77
1.244.....	99.68	99.67	1.330.....	99.72	99.69
1.553.....	99.62	99.59	1.663.....	99.60	99.61
2.178.....	99.41	99.42	2.328.....	99.46	99.46
3.111.....	99.18	99.18	3.326.....	99.23	99.22
3.716.....	98.96	99.02	3.991.....	99.06	99.07
$\text{NH}_4\text{Cl}$			$R=100-0.169\ m$		
0.7297.....	99.87	99.88	0.427.....	99.81	99.85
1.277.....	99.76	99.78	1.110.....	99.63	99.62
2.183.....	99.62	99.63	1.655.....	99.45	99.44
2.682.....	99.58	99.55	2.752.....	99.09	99.07
2.925.....	99.53	99.51	3.300.....	98.88	98.88
3.390.....	99.44	99.43	$\text{Pb}(\text{C}_2\text{H}_3\text{O}_2)_2$		
3.386.....	99.42	99.43			
6.78.....	98.85	98.85			
			1.0.....	100.03	
			2.0.....	100.04	
			5.0.....	100.11	

For purposes of analysis of crude products it is necessary to know the effect of acetates of lead upon the rotation of sucrose. Bates and Blake<sup>32</sup> have shown that basic lead acetate causes at low concentration a diminution of the rotation and at high concentration an increase. We have made similar measurements on the effect of normal lead acetate. As shown in Table 11, the normal acetate has an effect which for ordinary purposes is quite negligible.

In a later discussion, page 171, we shall show that ammonium chloride may be a serviceable reagent to add to the solution for direct polarization. This arises from the fact that ammonia is particularly suitable for neutralizing the hydrochloric acid in the invert polarization. We, therefore, determined carefully the effect of ammonium chloride on the rotation of sucrose. The results are assembled in Table 11.

<sup>32</sup> B. S. Bulletin, 3, p. 195; 1907.

The rotations of normal sucrose solutions in the presence of neutral salts are expressed by the following formulas:

$$R_{\text{NaCl}} = 100 - 0.265 m \quad (22)$$

$$R_{\text{K}_2\text{C}_2\text{O}_4} = 100 - 0.234 m \quad (23)$$

$$R_{\text{CaCl}_2} = 100 - 0.339 m \quad (24)$$

$$R_{\text{NH}_4\text{Cl}} = 100 - 0.169 m \quad (25)$$

where  $m$  represents the number of grams of salt present in the solution.

The precision which may be expected is illustrated by the agreement between the observed and calculated rotations shown in Table 11.

## VI. VALUE OF THE CLERGET DIVISOR IN THE PRESENCE OF ACIDS AND SALTS OF VARYING CONCENTRATION

### 1. INVERSION BY ACID OF VARYING CONCENTRATION

We have described on page 155 measurements of the influence of hydrochloric acid on the rotation of invert sugar. In those experiments a varying quantity of acid was added to invert sugar solutions which were previously prepared by means of invertase. In the method now to be described pure sucrose was inverted by acid of varying concentrations, each experiment furnishing an absolute and independent value. A quantity of pure sucrose was weighed in the volumetric flask and dried as previously described. It was dissolved, acidified, and inverted under the proper conditions of time and temperature stated on page 138. Its rotation was observed with all the precautions that were exercised in our most careful measurements. The results are summarized in Table 12. The rotations are expressed by the equation

$$R = 42.00 - 0.125 C = -42.00 - 0.5407 m = -42.00 - 1.972 N \quad (26)$$

in which  $C$  is the number of ml of 6.34 N HCl,  $m$  the number of grams of anhydrous HCl, and  $N$  the normality with respect to acid in 100 ml of the polarized solution.

TABLE 12.—Measurements of the Rotations of Solutions Inverted by Hydrochloric Acid of Varying Concentrations

Experiment No.	Weight of sugar (air, brass weights)	Volume of solution	Acidity of final solution	Method of inversion	Observed rotation		Temperature of observation	Rotation corrected for concentration and temperature	Calculated, $R = -42.00 - .5407 m$ ( $m = \text{grams HCl in 100 ml}$ )
					Observer J	Observer G			
	g	ml	N		°S	°S	°C	°S	S°
(From Table 8)			0.634					43.25	43.25
Expt. No. 9 . . . .	15.792	100.183	.317	20 minutes at 60° C	19.86 <sub>0</sub>	19.84 <sub>3</sub>	20.13	42.62	42.63
10 . . . .	16.451	100.000	.2012	35 minutes at 60° C	20.63 <sub>0</sub>	20.61 <sub>0</sub>	20.16	42.44	42.40
11 . . . .	15.822	100.185	.0804	32 minutes at 70° C	19.58 <sub>0</sub>	19.58 <sub>0</sub>	20.11	42.11	42.16
12 . . . .	15.942	100.183	.0804	100 minutes at 60° C	19.76 <sub>0</sub>	19.75 <sub>1</sub>	20.11	42.15	

## 2. DISCUSSION OF RESULTS

For the purposes of the present paper, two important conclusions may be reached from the measurements summarized in Tables 9 and 12 and Fig. 2. In the first place, let us compare the results obtained as described on page 165 and Table 12 with those described on page 155 and Table 9 relating to the effect of varying concentrations of acid on the rotation of invert sugar. In the earlier-described measurements which were made by adding acid to solutions already inverted by invertase, the value  $-43.25$  was used as the point of reference on which all the other determinations were based. None of these solutions was heated in the presence of acid, nor did any remain exposed to the action of acid longer than the necessary time of observation. Hence the question of the decomposition of the invert sugar was entirely eliminated. The curve on which this series was plotted, when extrapolated to zero concentration of acid, gave  $-42.00^\circ$  as the rotation of invert sugar in aqueous solution, when 13 g of sucrose was inverted and the invert polarization multiplied by 2. The values in Table 12, which we are now considering, were obtained by starting with sucrose and inverting with acid of varying strength. If this series is plotted in the same way and extrapolated to zero concentration of acid, it gives identically the same value for the rotation in pure aqueous solution. Now, this coincidence of the two curves, in one of which we know that no decomposition of



invert sugar had occurred, necessitates that in the other also no decomposition is involved. We have previously selected our conditions of inversion so that no decomposition should occur after the completion of the inversion. We may now conclude that no measureable decomposition occurs during the inversion.

If the argument presented above is valid, there seems to be no occasion for the substitution of organic acids for hydrochloric acid in order to avoid the decomposition of invert sugar.

The second interesting conclusion to be drawn from the curves, representing the variation with concentration of acid, relates to the value  $-42.00$  for the rotation of pure invert sugar in aqueous solution. The best method of obtaining the rotation of a pure invert sugar solution is by the use of invertase. Such a solution is free from any suspicion of decomposition products; and its rotation, corrected for the rotation of invertase itself, may be introduced into our curves at the point of zero concentration of acid to corroborate in a very striking manner the validity of our value  $-43.25$  and all intervening points. Fortunately we have available the result obtained by use of invertase. C. A. Browne<sup>33</sup> has carefully determined the invertase value for use in analytical determinations.

He reports that the rotation of 13 g of sucrose in 100 ml multiplied by 2 is  $-42$ . Inasmuch as he uses it in analyses of known solutions to two decimal places, he tacitly claims that it is accurately  $-42.00$ . This rotation is based on the Herzfeld-Schönrock standardization of the sugar scale. If converted to the scale proposed by Bates and Jackson the value becomes  $-42.03$ . This, as shown above, is within  $0.03$  of the polarization which we obtained by starting at  $-43.25$  and determining the slope of the curve by two different methods. It is interesting to note that if we start with Herzfeld's result  $-42.66$  and extrapolate by means of the slope of our acid-concentration curves we arrive at the value  $-41.4$ , which is hopelessly at variance with Browne's  $-42.00$ .

In proposing a modification of Clerget's original procedure Browne uses 5 ml of concentrated HCl in 55 ml of solution. Since his work was a report to the Association of Official Agricultural Chemists, he presumably used the 38.8 per cent acid, which the association prescribes. If this be true, his concentration of acid was 9.09 ml in 100 ml and the concentration of sucrose was 23.64

<sup>33</sup> J. Assoc. Off. Agr. Chem., 11, p. 134; 1916. Hudson reported the value  $-31.7$ . We have selected Browne's value because he verified it by a series of analyses of known mixtures.

g in 100 ml. He reports that the rotation of the inverted solution is  $-34.^{\circ}9$ . This value corrected to 13 g of sucrose is  $-34.^{\circ}19$  on the Herzfeld-Schönrock scale or  $-34.22$  on the Bates-Jackson scale. If we calculate the value of the rotation of 13 g of inverted sucrose in the presence of 9.09 ml of 38.8 per cent or 18.18 ml of 6.34 N HCl from our equation

$$R = 42.00 - 0.125 C \quad (16)$$

we obtain  $-42.00 - 0.125 \times 18.18 = -44.27$  or at  $20^{\circ} - 34.^{\circ}27$  S. This agreement with Browne's value  $-34.22$  is fairly satisfactory. In describing his procedure Browne merely states that he used 5 ml of concentrated hydrochloric acid. If he used the commercial chemically pure acid, it was undoubtedly less concentrated than the 38.8 per cent which the association prescribes. The possible variation in strength of acid would be sufficient to account for the difference of  $0.^{\circ}05$  S between our calculated and his observed values.

Steuerwald<sup>34</sup> proposed a modification of the Clerget method, in which the inversion is accomplished at room temperature by adding 30 ml of hydrochloric acid of 1.10 specific gravity. In lieu of a more definite specification we assume that this is specific gravity referred to water at  $20^{\circ}$  C. This specific gravity is equivalent to a density at  $20^{\circ}$ , referred to water at  $4^{\circ}$ , of 1.0981, which is the density of 6.045 N HCl. Steuerwald added to his sucrose solution 30 ml of this acid, which is equivalent to 28.59 ml of the 6.340 N HCl, upon which our formula is based. Substituting 28.59 ml in our formula, we obtain

$$R = -42.00 - 0.125 \times 28.59 = -45.57$$

Steuerwald reports the value  $-45.54$  on the Herzfeld-Schönrock scale, which is equivalent to  $-45.58$  on the Bates-Jackson scale.

This remarkably close agreement is, perhaps, somewhat fortuitous, inasmuch as Steuerwald did not specify the concentration of his acid solution with corresponding precision.

If, as now seems probable, the relation between rotation and concentration of acid is strictly linear between the concentrations of acid which we have in consideration, Browne's results for 5 ml of 38.8 per cent HCl in 55 ml or 9.09 ml in 100 ml ( $-44.22$ ) and for zero ml HCl ( $-42.03$ ) may be interpolated to give the rotation in

<sup>34</sup> *Archief*, 21, p. 831, 1913; *Int. Sug. J.*, 15, p. 489, 1913.

the presence of 5 ml of 38.8 per cent acid in 100 ml. The interpolated value differs from ours by but  $0.001$  S. Similarly, if we interpolate between the invertase value  $-42.03$  and that of Steuerwald ( $-45.58$ ) in presence of 28.59 ml of 6.34 N HCl, we obtain for 10 ml of 6.34 N HCl the rotation  $-43.27$ , which differs from ours by only  $0.002$  S. Thus, in recapitulation we find that our formula expressing the rotation of invert sugar and its variation with the concentration of hydrochloric acid correlates very closely the four values obtained in three different investigations. These values have hitherto been isolated empirical quantities, bearing no apparent relation to each other. We may conclude that the accessory data furnish a strong corroboration of  $-43.25$  S for the rotation of invert sugar in the presence of 0.634 N HCl. Herzfeld's value  $-42.66$  can in no way be reconciled with these experiments.

### 3. VALUE OF THE DIVISOR IN THE PRESENCE OF NEUTRALIZED ACID

(a) NEUTRALIZATION BY SODIUM HYDROXIDE.—It is frequently necessary that the solution for the invert polarization shall be neutral in reaction in order that optically active impurities shall have a constant rotation in both polarizations. This is notably the case with many amino compounds which are present in beet juices. For example, asparagine has a specific rotation of  $-6$  in neutral solution and  $+34$  in acid solution. Obviously, such an impurity will constitute a serious source of inaccuracy unless both polarizations are in neutral solution.

In suggesting the neutralization method, Browne<sup>35</sup> states that the effect of salt is small compared with that of acid. We find, very much to the contrary, that the effect of salt is about 1.6 times as great as that of a chemically equivalent quantity of hydrochloric acid. In order that the direct and invert polarization may be made under similar conditions, Saillard<sup>36</sup> suggested the addition of an equivalent quantity of salt to the solution to be polarized directly. Browne suggested that the Clerget constant be determined by making parallel analyses on pure sucrose. Inasmuch as the neutralization method is undoubtedly of value in many instances and should be as reproducible as the acid method, we considered that the value of the divisor in this modification required accurate evaluation. Consequently, we have made measurements

<sup>35</sup> Handbook of Sugar Analysis, p. 271; first thousand.

<sup>36</sup> Eighth Int. Cong. Applied Chem. Communications, 25, p. 541.

of the rotation in the presence of neutralized hydrochloric acid in four concentrations.

The sugar in the volumetric flask was inverted by HCl solutions of varying concentration under the conditions of time and temperature described on page 138. The acid was carefully measured by use of an accurate pipette. Solutions of sodium hydroxide of approximately the concentration of the respective acid solutions were prepared and their values in terms of the acid solutions carefully determined by use of methyl orange as indicator. If done in this manner, the equivalent volume of alkali may be determined once for all, and no titration of the inverted sugar solution is required. After completion of the inversion, the solution was cooled to approximately the temperature of polarization and the equivalent volume of alkali added from a burette. It was found necessary to add the alkali slowly and with continual shaking in order that the invert sugar should at no time be exposed to the strong alkali.

Unless the analyst has time and facilities for performing this neutralization with care, it is much preferable to polarize the acid solution, for a very slight alkalinity is sufficient to cause a rapid destruction of the invert sugar. It is safer to leave the solution very slightly acid.

After neutralization, the flask containing the solution was immersed in the thermostat, the solution made to volume, and polarized as usual. The results of these measurements are summarized in Table 13 and plotted in Fig. 2, page 160. As appears in the table, a solution of invert sugar which would polarize  $-43.25$  in acid solution, polarizes  $-44.00$  if the acid is neutralized by sodium hydroxide. The relation between rotation and weight of salt is expressed by the equation

$$R = -42.00 - 0.540 m, \quad (18)$$

in which  $m$  is the number of grams of salt in 100 ml of solution. This formula was obtained previously to these final measurements by measuring the effect of salt added to invert sugar solutions.

It will be noticed that the rotation of the inverted solution is linear with the varying concentrations of salt and, if extrapolated to zero concentration of salt, becomes  $-42.00$ , which is exactly the value obtained quite independently with hydrochloric acid.

TABLE 13.—Measurements of the Rotations of Solutions Inverted by Hydrochloric Acid and Neutralized by Sodium Hydroxide

Ex- peri- ment No.	Weight of sugar (air, brass weights)	Volume of solu- tion	Con- centra- tion of salt in final solution	Nor- mality of salt	Time of inver- sion; bath at 60°	Observed rotation		Tem- pera- ture of ob- serva- tion	Rotation correct- ed for tempera- ture and centra- tion	Mean correct- ed rota- tion	Calculated, $R = -42.00$ $-0.540\ m$ ( $m$ = grams NaCl per 100 ml)
						Ob- server J	Ob- server G				
	g	ml	g/100 ml	N	Min- utes	°S	°S	°C	°S	°S	°S
14	15.795	100.183	3.741	0.6399	9	-20.74 <sub>9</sub>	-20.73 <sub>1</sub>	20.08	-44.04 <sub>5</sub>	-44.02	-44.02
15	15.786	100.000	3.741	.6399	9	-20.71 <sub>7</sub>	-20.71 <sub>9</sub>	20.14	-43.99 <sub>7</sub>		
				.634							-44.00
16	23.131	100.000	2.945	.5038	11½	-28.68 <sub>9</sub>	-28.67 <sub>9</sub>	24.13	-43.60	-43.60	-43.59
17	15.899	100.000	1.870	.3199	20	-20.28 <sub>8</sub>	-20.32 <sub>9</sub>	20.11	-43.07 <sub>2</sub>	-43.01	-43.01
18	15.810	100.000	1.870	.3199	20	-20.12 <sub>7</sub>	-20.15 <sub>3</sub>	20.05	-42.95 <sub>5</sub>		
19	15.774	100.183	.4703	.0804	100	-19.60 <sub>3</sub>	-19.62 <sub>7</sub>	20.06	-42.24	-42.24	-42.25

(b) NEUTRALIZATION BY AMMONIUM HYDROXIDE.—In a number of the foregoing experiments the alkali was accidentally added in a very slight excess, which was in no instance greater than would correspond to the ordinary errors of titration. In these experiments, which are not reported, the decomposition of the invert sugar was so great that the validity of the result was completely destroyed. Obviously, the use of sodium hydroxide as a reagent in routine analysis is attended with great danger. We therefore determined the relative merits of sodium and ammonium hydroxide by making up solutions of invert sugar, 0.04 N with respect to the reagents, and observing the respective rates of decomposition at the temperature of the laboratory. The sodium hydroxide solution decreased regularly with time until by the following morning it had retained but two-thirds of its original rotary power. The ammonia solution, on the other hand, remained quite unaffected during the whole course of the observation. This comparison was so decisively in favor of ammonia as the neutralizing reagent that we decided to substitute it for sodium hydroxide in the proposed method of analysis.

We therefore determined the value of the Clerget divisor in the presence of 0.634 N  $\text{NH}_4\text{Cl}$  (3.392 g per 100 ml) by inverting with 10 ml of 6.34 N HCl as usual and neutralizing with ammonia. The exact volume of ammonia required was determined by previous titration. The results of the measurements are assembled in Table 14. As shown in the table, twice the rotation of 13 g of sucrose inverted and neutralized by ammonia is  $-33.91$  at  $20^\circ\text{C}$ .

TABLE 14.—Measurements of the Rotations of Solutions Inverted by 0.634 N Hydrochloric Acid and Neutralized by Ammonium Hydroxide

Experiment No.	Weight of sugar (air, brass weights) in 100 ml of solution	Observed rotation		Temperature of observation	Rotation corrected for temperature and concentration
		Observer J	Observer G		
	g	°S	°S	°C	°S
1.....	15.8518	20.72 <sub>1</sub>	20.73 <sub>0</sub>	20.23	43.92 <sub>3</sub>
2.....	15.6113	20.46 <sub>3</sub>	20.47 <sub>5</sub>	20.03	43.93 <sub>3</sub>
3.....	15.5662	20.33 <sub>2</sub>	20.34 <sub>2</sub>	20.14	43.86 <sub>3</sub>
4.....	15.5346	20.34 <sub>9</sub>	20.36 <sub>5</sub>	20.00	43.90 <sub>7</sub>
Mean.....					43.91

## VII. ANALYTICAL PROCEDURE

## 1. ELIMINATION OF AN ERROR CAUSED BY CHANGED CONCENTRATION OF INVERT SUGAR EXISTING AS AN IMPURITY

An error in the prevailing methods of Clerget analysis which has hitherto apparently been overlooked is due to the fact that the specific rotation of invert sugar changes considerably with a change in concentration. In Herzfeld's modification the normal weight of substance is contained in 100 ml of solution for the direct polarization, while for the invert polarization the half-normal weight is contained in 100 ml. For illustration let us suppose, as an extreme case, that pure invert sugar is subjected to the Clerget analysis. We ought to obtain zero sucrose, but, owing to the change of rotation with concentration, we would actually find  $-0.7$  per cent sucrose if all other sources of error were eliminated. This fact may be shown by selecting from the usual Clerget concentration tables the rotations of invert sugar at the normal and half-normal concentrations, viz:

$$S = \frac{-33.54 + 32.66}{132.66} = \frac{-.88}{132.66} = -0.7 \text{ per cent}$$

In such a case as this it is very necessary that the concentration of invert sugar shall be the same in both polarizations.

Quite another question arises if the invert sugar is one constituent of a sucrose-invert sugar mixture. In such a case, even if total volumes of solution remain constant, the invert sugar is at a relatively low concentration in the direct polarization, and at relatively high concentration in the invert polarization when all the sucrose has become invert sugar. The problem, then, is to

determine whether the original relatively small quantity of invert sugar produces the same rotation in the presence of sucrose that it does if all the sucrose is inverted. In the present state of our knowledge of the rotations of sugar mixtures, this could only be ascertained by experiment.

A stock solution of invert sugar was prepared from an approximately normal sucrose solution. Twenty cubic centimeters were pipetted into a 100 ml flask and made to volume with water. The solution polarized  $-6^{\circ}.11$ . Another portion of 20 ml was pipetted into a 100 ml flask, 19.500 g of sucrose added, and the flask filled to volume with water. This solution polarized  $+68^{\circ}.46$ . If the rotation of the 19.5 g of sucrose be taken as  $+75^{\circ}.00$ , the invert sugar rotated  $-6^{\circ}.54$ . Finally the stock solution of invert sugar itself read  $-33^{\circ}.13$ . Since we may consider that we took 100 ml of the stock solution for this last polarization, 20 ml of it produced one-fifth of the rotation of the total solution, or  $-6^{\circ}.62$ . It thus appears that this quantity of invert sugar, the same in the three polarizations, produces about the same rotation in the presence of sucrose as it does if the sucrose is inverted and quite a different rotation if diluted with water. We have found in a series of analyses of known solutions in which invert sugar is added as an impurity, that the rotation lacks constancy by about the amount indicated in this experiment. On the other hand, we find that the value of the divisor must be corrected for concentration by the same amount that it would be in pure aqueous solution. Our conclusions in this respect must be considered empirical, since the problem of the rotation of mixtures is too large a one for a complete solution at this time. Obviously the experiment cited is inadequate in scope and the question of this concentration effect must be considered an outstanding source of uncertainty. For the present we suggest that if the sample to be analyzed contains invert sugar, both polarizations be made at the same concentration of total substance. Fortunately, this involves but slight complication in the analytical procedure.

## 2. A CONVENIENT AND ACCURATE METHOD OF DILUTING TO ONE-TENTH GREATER VOLUME

The usual method of diluting a solution to one-tenth greater volume requires the use of a flask with two graduation marks, the neck being necessarily elongated or enlarged. This doubles the chance of volumetric error. There is also the possibility that the enlarged neck may not drain perfectly and a resulting error

be caused by the adhering liquid. Moreover, it is a time consuming operation to fill the flask accurately to the lower graduation mark.

In the method here proposed we have made use of the ordinary 100 ml flasks with the single graduation mark. The neck of the flask may be as small as desired. We have had made for the purpose of this research a number of pipettes which have been calibrated to deliver 90.91 ml of liquid. This volume delivered into a 100 ml flask and made to volume is then exactly one-tenth diluted. It may be said in general that the delivery from a pipette is one of the most accurate methods of taking a definite volume. It is under favorable conditions reproducible to a few thousandths of a milliliter. Even with a pipette of rapid delivery the volume may easily be reproduced within 0.01 ml. The only essential conditions are that the pipette shall be clean and shall be manipulated in exactly the same manner in which it was calibrated. It is preferable that the analyst should calibrate his own pipettes. The odd volume 90.91 ml offers no greater difficulty to the glass blower than the usual sizes. Obviously a pipette delivering 45.45, or more exactly, 45.455 ml may be used in combination with a 50 ml flask.

### 3. EVALUATION OF THE CLERGET DIVISOR

The numerical value of the Clerget divisor represents the algebraic difference between the rotations of sucrose before and after inversion. It is composed of two quite distinct quantities, which must be considered separately—namely, the positive constituent or the rotation of sucrose and the negative constituent or the rotation of the inverted sucrose—each rotation being measured under such conditions that other optically active substances have the same value in both polarizations.

(a) THE POSITIVE CONSTITUENT.—In aqueous solution the normal weight of sucrose has a rotation of  $100^{\circ}$  S at  $20^{\circ}$  C. If, therefore, the nature of the substance does not require the addition of reagents, 100 is the value of the positive constituent if the observations are made at  $20^{\circ}$  C. If at higher temperature, this figure must be diminished  $0^{\circ}.03$  S for each degree above  $20^{\circ}$  C, it being understood that the solution is made to volume and the quartz wedges are maintained at this same temperature. In the present discussion the rotations of less than normal concentrations are considered proportional to the concentration.



We have previously shown that if reducing sugars of approximately the composition of invert sugar are present, it is necessary, in order that their rotation shall be constant, to add a neutral salt to the solution to be polarized directly. In the special case in which 5 ml of 38.8 per cent hydrochloric acid is used to invert the sugar the quantity of sodium chloride required is 2.315 g in 100 ml of solution. This diminishes the rotation of sucrose to the value of 99.38, which thus becomes the positive constituent. If potassium oxalate is selected, the weight to be added is 2.451 g and the positive constituent becomes 99.425. The corresponding values for calcium chloride are 1.761 g and 99.40.

In general, if  $m$  grams of anhydrous hydrochloric acid are contained in 100 ml of the invert polarization, the weights of the salts to be added to the direct polarization are given by the equations:

$$(a) \ 1.001 \ m = \text{wt. NaCl} \quad (27)$$

$$(b) \ 1.060 \ m = \text{wt. K}_2\text{C}_2\text{O}_4 \quad (28)$$

$$(c) \ 0.762 \ m = \text{wt. CaCl}_2 \quad (29)$$

The corresponding positive constituents are then:

$$(a) \ R = 100 - 0.27 \ m \quad (30)$$

$$(b) \ R = 100 - 0.25 \ m \quad (31)$$

$$(c) \ R = 100 - 0.26 \ m \quad (32)$$

In method II, which will be described later and which is of perfectly general application, the inverted solution is neutralized by ammonia, and a quantity of ammonium chloride equal in weight to the neutralized acid is added to the solution for direct polarization. In the special case in which 5 ml of 38.8 per cent acid is used for inversion the weight of ammonium chloride is 3.392 and the positive constituent is 99.43.

In general, if the weight of the ammonium chloride is  $m$  grams, the positive constituent is:

$$R = 100 - 0.169 \ m \quad (21)$$

(b) THE NEGATIVE CONSTITUENT.—The value of the negative constituent varies markedly according to temperature, concentration of sugar, and quantity of added reagents. Considering first the rotation of 13 g of invert sugar in 100 ml, observed at 20° C and multiplied by 2, we may assign the proper values with respect to the reagents which are present. If 5 ml of 38.8 per cent HCl,

or 10 ml of 6.34 N HCl, are used to invert the sugar, the negative constituent is  $-33.25$ . When  $C$  ml of 6.34 N HCl are used, it is

$$R_{\text{HCl}} = -32.00 - 0.125 C \quad (16)$$

If the 10 ml of 6.34 N HCl acid is neutralized by ammonia the value becomes  $-33.91$ . When  $C$  milliliters of 6.34 N HCl are used, it becomes:

$$R_{\text{NH}_4\text{Cl}} = -32.00 - 0.191 C = -32.00 - 0.563 m, \quad (33)$$

in which  $m$  represents the grams of  $\text{NH}_4\text{Cl}$  in 100 ml of inverted solution.

The influence of the three variables may be expressed by the two following formulas:

$$R_{\text{HCl}} = -41.12 - 0.125 C - 0.0676 m + 0.5 t \quad (34)$$

$$R_{\text{NH}_4\text{Cl}} = -41.12 - 0.191 C' - 0.0676 m + 0.5 t \quad (35)$$

in which  $C$  represents the number of milliliters of 6.34 N HCl in 100 ml of solution,  $C'$  the number of milliliters of 6.34 N HCl neutralized by  $\text{NH}_4\text{OH}$ ,  $m$  the grams of sucrose which were contained in the invert solution before inversion, and  $t$  the centigrade temperature.

(c) THE COMBINED CONSTITUTENTS.—The positive and negative constituents may be combined to accord with the method by which the respective polarizations are made. For the sake of definiteness, we have suggested four methods, which are described in detail below. The value of the divisor in each instance has been computed for one concentration of hydrochloric acid, namely, 10 ml of 6.34 N HCl. The basic values and the influence of temperature and concentration are included in Tables 16 to 19, which are appended to the descriptions of the respective proposed methods.

As we have shown previously, the analyst may vary his concentration of acid or may neutralize his invert solution with sodium hydroxide, but must select the proper values to correspond with the procedure determined upon. In selecting the method, the analyst must bear in mind the essential principle that the rotation of all substances except sucrose must be kept constant in the two polarizations. We assemble below for ready reference the equations from which the value of the divisor may be computed.

1		2	
$R = 100.00$	(36)	$R = -32.00$	(37)
$R_{\text{NaCl}} = 100 - 0.265 m$	(22)	$R_{\text{HCl}} = -32.00 - 0.5407 m$	(17)
$R_{\text{K}_2\text{C}_2\text{O}_4} = 100 - 0.234 m$	(23)	$R_{\text{NaCl}} = -32.00 - 0.540 m$	(18)
$R_{\text{CaCl}_2} = 100 - 0.339 m$	(24)	$R_{\text{NH}_4\text{Cl}} = -32.00 - 0.563 m$	(21)
$R_{\text{NH}_4\text{Cl}} = 100 - 0.169 m$	(25)		

The algebraic difference between any equation in column 1 and any in column 2 gives the basic value of the divisor. This must be corrected for concentration and temperature by the equation

$$\text{Correction} = +0.0676 (m - 13) - \frac{t}{2} \quad (38)$$

in which  $m$  is the number of grams of sucrose taken for the invert polarization and  $t$  is the centigrade temperature.

#### 4. THE RAFFINOSE FORMULA

Creydt<sup>37</sup> has shown that, in the absence of other optically active substances, sucrose and raffinose in mixtures can be estimated by the Clerget method. This estimation depends upon the fact that for two unknown quantities two equations are sufficient. One of these equations states that the direct polarization is the sum of the rotations of the two constituents, and the other states that the invert polarization is the sum of the rotations of the products of hydrolysis. In the presence of invert sugar, the raffinose formula is inapplicable. In the absence of invert sugar, "raffinose" in beet products is a rough measurement of the positively rotating substances which interfere with the crystallization of sugar, but does not necessarily represent the true raffinose content.

Creydt's derivation of the raffinose formula is given concisely in Browne's Handbook<sup>38</sup> of Sugar Analysis, and the equations are reproduced partially from that source with the new values for the rotation of invert sugar substituted. Let the analysis be conducted at 20° C. The direct polarization is then

$$P = S + 1.852 R \quad (39)$$

$P$  is the direct reading,  $S$  per cent sucrose, and  $R$  per cent raffinose. Let the invert solution contain 13 g of sucrose inverted and the invert reading in the presence of 0.634 N HCl be multiplied by 2. The invert reading is

$$P' = -0.3325 S + 0.9490 R, \quad (40)$$

<sup>37</sup> Zeit. Ver. Zuckerind, 37, p. 153; 1887.

<sup>38</sup> Page 282.

whence, eliminating  $R$  from equations (39) and (40), we obtain

$$S = \frac{0.5124}{0.8449} \frac{P - P'}{1} \quad (41)$$

and from (39)

$$R = \frac{P - S}{1.852} \quad (42)$$

If the inverted solution is neutralized exactly by  $\text{NH}_4\text{OH}$ , equation (40) becomes

$$P'' = -0.3391 S + .9490 R \quad (43)$$

whence

$$S = \frac{0.5124}{0.8515} \frac{P - P''}{1} \quad (44)$$

The above equations are strictly applicable only when the analysis is conducted at  $20^\circ$  and the invert solution contains 13 g of inverted sugar. The following are applicable for various temperatures and concentrations:

$$S = \frac{P (0.4724 + 0.002 t) - P'}{0.8961 + 0.000676 m - 0.003 t} \quad (45)$$

$$R = \frac{P - S}{1.852} \quad (42)$$

The equation, when the inverted solution is neutralized by  $\text{NH}_4\text{OH}$ , follows:

$$S = \frac{P (0.4724 + 0.002 t) - P''}{0.9027 + 0.000676 m - 0.003 t} \quad (46)$$

in which  $m$  represents grams of sucrose taken for the invert solution. The temperature corrections take into consideration only the invert polarization. If the temperature coefficient of the rotation of sucrose is included, equation (45) becomes (47), and (46) becomes (48). The temperature coefficient of the direct polarization of raffinose is not as yet known.

$$S = \frac{(1 + 0.0003 (t - 20)) (P' - P (0.4724 + 0.002 t))}{0.8961 + 0.000676 m - 0.003 t} \quad (47)$$

$$S = \frac{(1 + 0.0003 (T - 20)) (P'' - P (0.4724 + 0.002 t))}{0.9027 + 0.000676 m - 0.003 t} \quad (48)$$

and

$$R = \frac{P - S}{1.852} \quad (42)$$

In constructing the equations (39) to (47) we are obliged to assume that the polariscopic constants of raffinose are precise and that the polarization of the hydrolyzed raffinose is the same in the presence of ammonium chloride as it is in hydrochloric acid. Since raffinose yields levulose on hydrolysis, this assumption is obviously not correct, but the error introduced when the raffinose content is but a few per cent is small compared with the error caused by the change of acidity on the nonsugars of the beet. The formula can be made precise when the constants of raffinose are further investigated.

## 5. GENERAL CONSIDERATIONS

In forming specifications for an analytical procedure on the basis of the foregoing experiments we have been partially guided by a desire not to complicate the method unduly. Fortunately, we have found it merely necessary to suggest a few simple precautions in order to insure reproducibility and to secure constancy of rotation for other substances than sucrose.

Considering alone the products involved in the sugar industry, we may base our discussion on the properties of the four constituents, sucrose, invert sugar, optically active nonsugars whose rotation is affected by a change in acidity, and all other nonsugars which are optically unaffected by the presence of acid. By the third group we mean particularly the impurities occurring in beet juices which change in rotation as the concentration of hydrogen ion changes. Such may or may not be present in cane juices, but, in any event, are usually small in amount and of low optical activity.

The complete specifications for the analytical methods should include a detailed description of the clarification procedure. Fortunately, a great advance in clarification has been made by Dr. W. D. Horne<sup>39</sup>, who suggested the addition of dry basic lead acetate after the solution to be analyzed has been made to volume. While Horne's method very largely eliminates the error caused by the volume of the precipitate, there remains to be specified the composition of the basic acetate of most effective basicity. From results which we have obtained, but which do not yet permit of generalization, we conclude that the basic portion of the lead enters more largely into the precipitate than the neutral portion. It thus appears that while the clarified filtrate usually contains an excess of lead, the latter is very nearly neutral in reaction. Since

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<sup>39</sup> J. Amer. Chem. Soc., 26, p. 186; 1904.

basic lead acetate solutions contain too low a concentration of hydroxyl ions to affect indicators, it is certainly without influence on the amino compounds of beet juices which are much less sensitive to the reaction of the solution than indicators are. We are, then, concerned (in the absence of invert sugar) with the effect of the clarifier upon the rotation of sucrose. Bates and Blake<sup>40</sup> have shown that basic lead acetate causes at low concentration a diminution of rotation of sucrose and at high concentrations an elevation. We have shown on page 164 that neutral lead acetate has an entirely negligible influence on the rotation of sucrose. Consequently, it is only necessary for the analyst to assure himself that his filtrate is not basic. In the present state of the clarification question the greatest safety lies in the use of the minimum quantity of the reagent. It is an open question whether it is permissible to add acetic acid to the direct polarization. An excess of acetic acid might influence in uncertain degree the rotation of the nonsugars. It is, therefore, obvious that our proposed procedures are provisional to the extent that the methods of clarification are provisional.

In Table 15 we have assembled the three variables which may be encountered in a sample requiring analysis. It is not intended to imply that analyses performed by the prevailing methods give results which are seriously in error. The table indicates only the effect of each item taken individually. The collective effect can only be measured by an extensive series of comparative analyses.

TABLE 15.—Summarizing Defects in Prevailing Methods of Clerget Analysis

[D. P.=direct polarization, made directly or acidified with acetic acid and made up to one-tenth greater volume. I. P.=invert polarization, 50 ml plus 25 ml water, or 75 ml made up to 100 ml]

Method of polarization	Sucrose	Invert sugar in original sample	Nonsugars of beet
D. P. of filtrate from lead clarification	Constant not reproducible; <sup>a</sup> 142.66 erroneous <sup>b</sup>	Seriously affected (1) by change of concentration <sup>c</sup> (2) I. P. elevated <sup>d</sup> by HCl	Affected by change of acidity
I. P. in HCl solution			
D. P. of filtrate from lead clarification	Constant not reproducible; <sup>a</sup> 142.66 erroneous <sup>b</sup>	Seriously affected (1) by change of concentration <sup>c</sup> (2) I. P. elevated by HCl <sup>d</sup> (3) I. P. elevated by de-leading agent <sup>e</sup>	Affected by change of acidity
I. P. de-leaded and read in HCl solution			
D. P. in acetic-acid solution	Constant not reproducible; <sup>a</sup> 142.66 erroneous <sup>b</sup>	Seriously affected (1) by change of concentration <sup>c</sup> (2) I. P. elevated by HCl <sup>d</sup> (3) I. P. elevated by de-leading agent <sup>e</sup> (4) D. P. elevated by depressing effect of acetic on invert sugar <sup>f</sup>	Affected by change of acidity
I. P. de-leaded and read in HCl solution			

<sup>a</sup> See p. 153.

<sup>b</sup> See p. 150.

<sup>c</sup> See p. 172.

<sup>d</sup> See p. 155.

<sup>e</sup> See p. 160.

<sup>f</sup> See p. 156.

<sup>40</sup> B. S. Bulletin, 3, p. 105; 1907 (Scientific Paper No. 52).

We have suggested four general methods from which the analyst may select the one best adapted to the nature of the material which is under examination. The second of these is of perfectly general application and requires no knowledge of the nature of the impurities in the substance to be analyzed. It may thus be used either for ordinary routine analysis or as a control to ascertain whether the other methods which have a more restricted applicability may be applied.

As alternatives to this method we have suggested three others, one of which, method III, is intended for use in beet products which are free from reducing substances, another, method IV, for use in cane products free from the optically active impurities whose rotation is a function of the hydrogen ion concentration, and another, method I, applicable to substances of high purity.

#### 6. GENERAL METHODS OF INVERSION

(a) Pipette 50 ml of the solution into a 100 ml flask, add 20 ml of water and 10 ml of hydrochloric acid,  $d_{40}^{20} 1.1029$ . Immerse in a water bath at 60°. Agitate the solution continually for about 3 minutes and allow it to remain in the bath for a total time of 9 minutes. Cool quickly.

(b) Pipette 70 ml of the solution into a 100 ml flask, add 10 ml of hydrochloric acid,  $d_{40}^{20} 1.1029$ , and proceed as in (a).

(c) Pipette 75 ml of the solution into a 100 ml flask, add 5 ml of hydrochloric acid,  $d_{40}^{20} 1.1918$ , and proceed as in (a).

(d) Pipette 75 cc of the solution, add 10 ml of hydrochloric acid,  $d_{40}^{20} 1.1029$ , and proceed as in (a), but allow the solution to remain a total time of 9.5 minutes.

(e) Pipette 70 ml or 75 ml of the solution into a 100 ml flask. Heat the solution to 65° C, add 10 ml of hydrochloric acid,  $d_{40}^{20} 1.1029$ , or 5 ml of acid  $d_{40}^{20} 1.1918$ . Mix and allow the solution to stand in the room for 15 minutes (Walker's method).

(f) Pipette 90.91 ml of the solution into a 100 ml flask. Heat to 65°, add 5 ml of 38.8 per cent HCl  $d_{40}^{20} 1.1918$ , mix by rotating the flask, and allow to remain on the laboratory table for 15 min-

utes. (The greater dilution of the acid is offset by the fact that a greater volume of solution cools more slowly.)

(g) Pipette 50 ml and add 20 ml of water, or pipette 70 ml of the solution, into a 100 ml flask, add 10 ml of acid  $d_{4}^{20^{\circ}} 1.1029$  and allow to remain 30.8 hours at  $20^{\circ}$ , 14.6 hours at  $25^{\circ}$ , 7.1 hours at  $30^{\circ}$ , 106 minutes at  $40^{\circ}$ , or 29 minutes at  $50^{\circ}$ . Cool quickly.

(h) Pipette 50 ml of the solution into a 100 ml flask, add 5 ml of acid  $d_{4}^{20^{\circ}} 1.1918$ , and allow to remain 21.2 hours at  $20^{\circ}$  or 10 hours at  $25^{\circ}$ .

## 7. METHOD I

(Applicable to pure sucrose, or to sucrose mixtures in which the impurities are unaffected optically by hydrochloric acid.)

Reagents: Hydrochloric acid  $d_{4}^{20^{\circ}} 1.1029$ , Brix 24.85, or hydrochloric acid  $d_{4}^{20^{\circ}} 1.1918$ , Brix 42.96.

Prepare a normal solution of the sample or a solution of such fractional normality as the nature of the substance and the sensibility of the saccharimeter will permit. Make to volume at the temperature at which the observations are to be made. If necessary, clarify by the addition of dry basic lead acetate and filter.

(If desired, the excess of lead may be removed at this point by the addition of pulverized potassium or sodium oxalate. It is, however, necessary that the whole filtrate be treated by the deleading reagent, since the latter exerts an effect upon the rotation of invert sugar which should be as far as possible offset by a similar effect on sucrose.) Polarize the solution to obtain the direct reading and, if necessary, correct to the value which would have been obtained if 26 g of the sample were taken in 100 ml of solution.

To obtain the invert polarization, invert as described on page 181, make to volume at the temperature at which the observations are to be made, and polarize. Multiply the observed polarization by the factor

$$\frac{\text{Volume of original solution containing 26 g of sample}}{\text{Volume of solution taken for invert polarization}}$$

The algebraic difference between the two polarizations corrected for dilution gives the value  $P - P'$ . From Table 16, under the column which designates the volume of the solution taken for the invert polarization, find the proper value of the Clerget divisor.



Subtract the temperature correction and divide  $P-P'$  by the corrected divisor. If the original solution was a fractional normality,  $P-P'$  must be multiplied by this fraction in order to select the proper value of the divisor. This is then divided into the corrected  $P-P'$ .

*Example 1.*—A solution of sucrose and dextrose made up to contain 2 x 26 gm of a sample in 200 ml, polarized +81.25. For the invert polarization 70 ml were taken, inverted and made up to 100 ml. The invert polarization  $\times 10/7$  was -24.86 at 20° C.  $P-P'$  was thus 106.11; the Clerget divisor, 133.35; and sucrose, 79.57.

(See also example 2, p. 185.)

TABLE 16.—Method I

[Sucrose=100° S; 13 g Invert sugar $\times 2$ = -33.°25 S]

$P-P'$	Volume of solution taken for invert polarization				Temperature corrections (to be subtracted)											
	50 ml $\times 2$	70 ml $\times \frac{10}{7}$	75 ml $\times \frac{4}{3}$	90.91 ml $\times \frac{11}{10}$												
133.97	-----	-----	-----	133.97	20.0	0.00	23.0	1.59	26.0	3.18	29.0	4.77	32.0	6.36		
133.69	-----	-----	133.69	-----	20.1	.05	23.1	1.64	26.1	3.23	29.1	4.82	32.1	6.41		
133.60	-----	133.60	-----	-----	20.2	.11	23.2	1.70	26.2	3.29	29.2	4.88	32.2	6.47		
133.25	133.25	-----	-----	-----	20.3	.16	23.3	1.75	26.3	3.34	29.3	4.93	32.3	6.52		
133	133.25	133.59	133.68	133.95	20.4	.21	23.4	1.80	26.4	3.39	29.4	4.98	32.4	6.57		
130	133.23	133.57	133.65	133.92	20.5	.27	23.5	1.86	26.5	3.44	29.5	5.04	32.5	6.63		
125	133.20	133.53	133.60	133.86	20.6	.32	23.6	1.91	26.6	3.50	29.6	5.09	32.6	6.68		
120	133.16	133.48	133.56	133.80	20.7	.37	23.7	1.96	26.7	3.55	29.7	5.14	32.7	6.73		
115	133.13	133.43	133.51	133.75	20.8	.42	23.8	2.01	26.8	3.60	29.8	5.19	32.8	6.78		
110	133.09	133.38	133.46	133.69	20.9	.48	23.9	2.07	26.9	3.66	29.9	5.25	32.9	6.84		
105	133.06	133.34	133.41	133.63	21.0	.53	24.0	2.12	27.0	3.71	30.0	5.30	33.0	6.89		
100	133.03	133.29	133.36	133.57	21.1	.58	24.1	2.17	27.1	3.76	30.1	5.35	33.1	6.94		
95	133.00	133.25	133.31	133.51	21.2	.64	24.2	2.23	27.2	3.82	30.2	5.41	33.2	7.00		
90	132.97	133.20	133.26	133.45	21.3	.69	24.3	2.28	27.3	3.87	30.3	5.46	33.3	7.05		
85	132.93	133.16	133.21	133.39	21.4	.74	24.4	2.33	27.4	3.92	30.4	5.51	33.4	7.10		
80	132.90	133.11	133.16	133.33	21.5	.80	24.5	2.39	27.5	3.98	30.5	5.57	33.5	7.16		
75	132.86	133.07	133.12	133.27	21.6	.85	24.6	2.44	27.6	4.03	30.6	5.62	33.6	7.21		
70	132.83	133.02	133.07	133.21	21.7	.90	24.7	2.49	27.7	4.08	30.7	5.67	33.7	7.26		
65	132.80	132.98	133.02	133.15	21.8	.95	24.8	2.54	27.8	4.13	30.8	5.72	33.8	7.31		
60	132.77	132.93	132.97	133.09	21.9	1.01	24.9	2.60	27.9	4.19	30.9	5.78	33.9	7.37		
55	132.73	132.88	132.92	133.03	22.0	1.06	25.0	2.65	28.0	4.24	31.0	5.83	34.0	7.42		
50	132.70	132.83	132.87	132.97	22.1	1.11	25.1	2.70	28.1	4.29	31.1	5.88	34.1	7.47		
45	132.66	132.79	132.82	132.91	22.2	1.17	25.2	2.76	28.2	4.35	31.2	5.94	34.2	7.53		
40	132.63	132.74	132.77	132.85	22.3	1.22	25.3	2.81	28.3	4.40	31.3	5.99	34.3	7.58		
35	132.60	132.70	132.72	132.79	22.4	1.27	25.4	2.86	28.4	4.45	31.4	6.04	34.4	7.63		
30	132.57	132.65	132.67	132.73	22.5	1.33	25.5	2.92	28.5	4.51	31.5	6.10	34.5	7.69		
25	132.54	132.61	132.62	132.67	22.6	1.38	25.6	2.97	28.6	4.56	31.6	6.15	34.6	7.74		
20	132.51	132.56	132.57	132.61	22.7	1.43	25.7	3.02	28.7	4.61	31.7	6.20	34.7	7.79		
15	132.47	132.51	132.52	132.55	22.8	1.48	25.8	3.07	28.8	4.66	31.8	6.25	34.8	7.84		
10	132.44	132.46	132.47	132.49	22.9	1.54	25.9	3.13	28.9	4.72	31.9	6.31	34.9	7.90		
5	132.40	132.41	132.42	132.43	23.0	1.59	26.0	3.18	29.0	4.77	32.0	6.36	35.0	7.95		

## 8. METHOD II

[General method applicable to all products]

Reagents: Hydrochloric acid  $d_{\frac{20^{\circ}}{4}} 1.1029$  (24.846 Brix); ammonium hydroxide solution, 5 to 6 N; solution of ammonium chloride containing 226 g per liter; pulverized potassium or sodium oxalate.

Ascertain by at least three concordant titrations in the presence of methyl orange the volume of the ammonia solution required to neutralize 10 ml of the hydrochloric acid.

Prepare the normal solution of the substance to be analyzed or a solution of such fractional normality as the nature of the material and the sensibility of the saccharimeter will permit. Clarify with the minimum quantity of dry basic lead acetate. Shake thoroughly and filter.

(If desired, the solution may at this point be freed from lead; but, if this is done, the deleading reagent must be added to the whole filtrate. Finely pulverized potassium oxalate in minimum quantity is added until precipitation is complete. Filter. If this procedure is omitted, the lead is precipitated satisfactorily by the chlorides added later.)

Pipette into two 100 ml flasks two equal volumes of the filtrate (50 ml, 70 ml, or 75 ml).

For the direct polarization, add to one portion 15 ml of the ammonium chloride solution or 3.392 g of dry ammonium chloride. Make to volume at the temperature at which the observations are to be made; filter, if necessary, and polarize.

For the invert polarization add to the other portion hydrochloric acid and invert by one of the methods described on page 181. Cool quickly. After the solution has become quite cold add from a burette during continual shaking the precisely determined volume of ammonia required to neutralize the acid. Adjust the temperature, make to volume, filter, if necessary, and polarize at carefully controlled temperature.

Multiply both polarizations by the factor—

$$\frac{\text{Volume of original solution containing 26 g of sample.}}{\text{Volume of solution taken for polarization.}}$$

The algebraic difference between the corrected polarizations gives  $P - P'$ . If the original filtrate contained 26 g in 100 ml, refer to Table 17, and under the column which designates the volume taken for the invert polarization find the value of the divisor. Apply the temperature correction and divide into  $P - P'$ . If the original solution contained a fraction of 26 g of the sample, multiply  $P - P'$  by this fraction before referring to Table 17. Divide into  $P - P'$ .

*Example 2.*—Twenty-six grams of a sample were dissolved in 300 ml of solution. Two 75 ml portions were taken, prepared for direct and invert polarization, respectively, and finally made up to 100 ml. The direct polarization multiplied by  $300/75=4$  proved to be 38.75. The invert polarization multiplied by 4 was  $-16.22$  at  $22.4^{\circ}\text{C}$ .  $P-P'$  was thus 54.97. Since the original sample was in  $1/3$  normal solution the actual concentration of sucrose was proportional to  $1/3 \times (P-P')$  or 18.32. Opposite 18.32 and under the column "75 cc taken" we find the divisor to be 132.63. This is diminished by 1.27 for the temperature correction to give 131.36 which divided into 54.97 gives 41.85 per cent sucrose.

(See also example 1, p. 183.)

TABLE 17.—Method II

[Sucrose + 3.392 g  $\text{NH}_4\text{Cl}$  =  $+99^{\circ}.43\text{ S}$ ; (13 g invert sugar + 3.392 g  $\text{NH}_4\text{Cl}$ )  $\times 2$  =  $-33^{\circ}.91\text{ S}$ ]

$P-P'$	Volume of solution taken for invert polarization				Temperature corrections (to be subtracted)											
	50 ml $\times 2$	70 ml $\times \frac{10}{7}$	75 ml $\times \frac{4}{3}$	90.91 ml $\times \frac{11}{10}$												
134.06	.....	.....	.....	134.06	20.0	0.00	23.0	1.59	26.0	3.18	29.0	4.77	32.0	6.36		
133.78	.....	.....	133.78	.....	20.1	.05	23.1	1.64	26.1	3.23	29.1	4.82	32.1	6.41		
133.69	.....	133.69	.....	.....	20.2	.11	23.2	1.70	26.2	3.29	29.2	4.88	32.2	6.47		
133.34	133.34	.....	.....	.....	20.3	.16	23.3	1.75	26.3	3.34	29.3	4.93	32.3	6.52		
133	133.34	133.68	133.77	134.04	20.4	.21	23.4	1.80	26.4	3.39	29.4	4.98	32.4	6.57		
130	133.32	133.66	133.74	134.01	20.5	.27	23.5	1.86	26.5	3.44	29.5	5.04	32.5	6.63		
125	133.29	133.62	133.69	133.95	20.6	.32	23.6	1.91	26.6	3.50	29.6	5.09	32.6	6.68		
120	133.25	133.57	133.65	133.89	20.7	.37	23.7	1.96	26.7	3.55	29.7	5.14	32.7	6.73		
115	133.22	133.52	133.60	133.84	20.8	.42	23.8	2.01	26.8	3.60	29.8	5.19	32.8	6.78		
110	133.18	133.47	133.55	133.78	20.9	.48	23.9	2.07	26.9	3.66	29.9	5.25	32.9	6.84		
105	133.15	133.43	133.50	133.72	21.0	.53	24.0	2.12	27.0	3.71	30.0	5.30	33.0	6.89		
100	133.12	133.38	133.45	133.66	21.1	.58	24.1	2.17	27.1	3.76	30.1	5.35	33.1	6.94		
95	133.09	133.34	133.40	133.60	21.2	.64	24.2	2.23	27.2	3.82	30.2	5.41	33.2	7.00		
90	133.06	133.29	133.35	133.54	21.3	.69	24.3	2.28	27.3	3.87	30.3	5.46	33.3	7.05		
85	133.02	133.25	133.30	133.48	21.4	.74	24.4	2.33	27.4	3.92	30.4	5.51	33.4	7.10		
80	132.99	133.20	133.25	133.42	21.5	.80	24.5	2.39	27.5	3.98	30.5	5.57	33.5	7.16		
75	132.95	133.16	133.21	133.36	21.6	.85	24.6	2.44	27.6	4.03	30.6	5.62	33.6	7.21		
70	132.92	133.11	133.16	133.30	21.7	.90	24.7	2.49	27.7	4.08	30.7	5.67	33.7	7.26		
65	132.89	133.07	133.11	133.24	21.8	.95	24.8	2.54	27.8	4.13	30.8	5.72	33.8	7.31		
60	132.86	133.02	133.06	133.18	21.9	1.01	24.9	2.60	27.9	4.19	30.9	5.78	33.9	7.37		
55	132.82	132.97	133.01	133.12	22.0	1.06	25.0	2.65	28.0	4.24	31.0	5.83	34.0	7.42		
50	132.79	132.92	132.96	133.06	22.1	1.11	25.1	2.70	28.1	4.29	31.1	5.88	34.1	7.47		
45	132.75	132.88	132.91	133.00	22.2	1.17	25.2	2.76	28.2	4.35	31.2	5.94	34.2	7.53		
40	132.72	132.83	132.86	132.94	22.3	1.22	25.3	2.81	28.3	4.40	31.3	5.99	34.3	7.58		
35	132.69	132.79	132.81	132.88	22.4	1.27	25.4	2.86	28.4	4.45	31.4	6.04	34.4	7.63		
30	132.66	132.74	132.76	132.82	22.5	1.33	25.5	2.92	28.5	4.51	31.5	6.10	34.5	7.69		
25	132.63	132.70	132.71	132.76	22.6	1.38	25.6	2.97	28.6	4.56	31.6	6.15	34.6	7.74		
20	132.60	132.65	132.66	132.70	22.7	1.43	25.7	3.02	28.7	4.61	31.7	6.20	34.7	7.79		
15	132.56	132.60	132.61	132.64	22.8	1.48	25.8	3.07	28.8	4.66	31.8	6.25	34.8	7.84		
10	132.53	132.55	132.56	132.58	22.9	1.54	25.9	3.13	28.9	4.72	31.9	6.31	34.9	7.90		
5	132.49	132.50	132.51	132.52	23.0	1.59	26.0	3.18	29.0	4.77	32.0	6.36	35.0	7.95		

## 9. METHOD III

[Beet products]

Reagents: Hydrochloric acid  $d_{\frac{20^{\circ}}{4^{\circ}}} 1.1029$  (24.85 Brix), ammonium hydroxide 5 to 6 N, pulverized potassium or sodium oxalate.

Ascertain accurately, with methyl orange as indicator, the volume of the ammonia solution required to neutralize 10 ml of the hydrochloric acid.

Prepare the normal solution or a solution of such fractional normality as the nature of the substance requires and make up to the volume of the flask at the temperature at which the observations are to be made. Clarify with the minimum quantity of dry basic-lead acetate and filter.

(If desired, the solution may at this point be freed from lead, but the deleading reagent must be added to the whole filtrate. Finely pulverized potassium oxalate in minimum quantity is added until precipitation is complete. Filter.)

The filtrate is polarized to obtain the direct reading.

To obtain the invert reading, pipette 70 ml into a 100 ml flask, add 10 ml of hydrochloric acid  $d_{\frac{20^{\circ}}{4^{\circ}}} 1.1029$ , and invert as described on page 181. Cool rapidly.

To the cold solution add the precisely determined volume of ammonia required exactly to neutralize the acid. Make to volume at the temperature at which the observations are to be made. Polarize.

Convert both readings to those of the normal solution by multiplying by the factor

$$\frac{\text{Volume of original solution containing 26 g of sample.}}{\text{Volume of solution taken for polarization.}}$$

The algebraic difference between the corrected polarizations gives the value  $P - P'$ . If the original solution contained 26 g in 100 ml, refer to Table 18, and under the column which designates the volume taken for the invert polarization find the value of the divisor. Apply the temperature correction and divide into  $P - P'$ . If the original solution was of fractional normality, multiply  $P - P'$  by this fraction before referring to Table 18. Divide into  $P - P'$ .

See examples 1 and 2 under Methods I and II.

TABLE 18.—Method III

[Sucrose = 100° S; (13 g invert sugar + 3.392 g  $\text{NH}_4\text{Cl}$ )  $\times 2 = -33^\circ.91$  S]

P—P'	Volume of solution taken for invert polarization				Temperature corrections (to be subtracted)											
	50 ml $\times 2$	70 ml $\times \frac{10}{7}$	75 ml $\times \frac{4}{3}$	90.91 ml $\times \frac{11}{10}$												
134.63	-----	-----	-----	134.63	20.0	0.00	23.0	1.59	26.0	3.18	29.0	4.77	32.0	6.36		
134.35	-----	-----	134.35	-----	20.1	.05	23.1	1.64	26.1	3.23	29.1	4.82	32.1	6.41		
134.26	-----	134.26	-----	-----	20.2	.11	23.2	1.70	26.2	3.29	29.2	4.88	32.2	6.47		
133.91	133.91	-----	-----	-----	20.3	.16	23.3	1.75	26.3	3.34	29.3	4.93	32.3	6.52		
133	133.91	134.25	134.34	134.61	20.4	.21	23.4	1.80	26.4	3.39	29.4	4.98	32.4	6.57		
130	133.89	134.23	134.31	134.58	20.5	.27	23.5	1.86	26.5	3.44	29.5	5.04	32.5	6.63		
125	133.86	134.19	134.26	134.52	20.6	.32	23.6	1.91	26.6	3.50	29.6	5.09	32.6	6.68		
120	133.82	134.14	134.22	134.46	20.7	.37	23.7	1.96	26.7	3.55	29.7	5.14	32.7	6.73		
115	133.79	134.09	134.17	134.41	20.8	.42	23.8	2.01	26.8	3.60	29.8	5.19	32.8	6.78		
110	133.75	134.04	134.12	134.35	20.9	.48	23.9	2.07	26.9	3.66	29.9	5.25	32.9	6.84		
105	133.72	134.00	134.07	134.29	21.0	.53	24.0	2.12	27.0	3.71	30.0	5.30	33.0	6.89		
100	133.69	133.95	134.02	134.23	21.1	.58	24.1	2.17	27.1	3.76	30.1	5.35	33.1	6.94		
95	133.66	133.91	133.97	134.17	21.2	.64	24.2	2.23	27.2	3.82	30.2	5.41	33.2	7.00		
90	133.63	133.86	133.92	134.11	21.3	.69	24.3	2.28	27.3	3.87	30.3	5.46	33.3	7.05		
85	133.59	133.82	133.87	134.05	21.4	.74	24.4	2.33	27.4	3.92	30.4	5.51	33.4	7.10		
80	133.56	133.77	133.82	133.99	21.5	.80	24.5	2.39	27.5	3.98	30.5	5.57	33.5	7.16		
75	133.52	133.73	133.78	133.93	21.6	.85	24.6	2.44	27.6	4.03	30.6	5.62	33.6	7.21		
70	133.49	133.68	133.73	133.87	21.7	.90	24.7	2.49	27.7	4.08	30.7	5.67	33.7	7.26		
65	133.46	133.64	133.68	133.81	21.8	.95	24.8	2.54	27.8	4.13	30.8	5.72	33.8	7.31		
60	133.43	133.59	133.63	133.75	21.9	1.01	24.9	2.60	27.9	4.19	30.9	5.78	33.9	7.37		
55	133.39	133.54	133.58	133.69	22.0	1.06	25.0	2.65	28.0	4.24	31.0	5.83	34.0	7.42		
50	133.36	133.49	133.53	133.63	22.1	1.11	25.1	2.70	28.1	4.29	31.1	5.88	34.1	7.47		
45	133.32	133.45	133.48	133.57	22.2	1.17	25.2	2.76	28.2	4.35	31.2	5.94	34.2	7.53		
40	133.29	133.40	133.43	133.51	22.3	1.22	25.3	2.81	28.3	4.40	31.3	5.99	34.3	7.58		
35	133.26	133.36	133.38	133.45	22.4	1.27	25.4	2.86	28.4	4.45	31.4	6.04	34.4	7.63		
30	133.23	133.31	133.33	133.39	22.5	1.33	25.5	2.92	28.5	4.51	31.5	6.10	34.5	7.69		
25	133.20	133.27	133.28	133.33	22.6	1.38	25.6	2.97	28.6	4.56	31.6	6.15	34.6	7.74		
20	133.17	133.22	133.23	133.27	22.7	1.43	25.7	3.02	28.7	4.61	31.7	6.20	34.7	7.79		
15	133.13	133.17	133.18	133.21	22.8	1.48	25.8	3.07	28.8	4.66	31.8	6.25	34.8	7.84		
10	133.10	133.12	133.13	133.15	22.9	1.54	25.9	3.13	28.9	4.72	31.9	6.31	34.9	7.90		
5	133.06	133.07	133.08	133.09	23.0	1.59	26.0	3.18	29.0	4.77	32.0	6.36	35.0	7.95		

## 10. METHOD IV

[Cane products]

(Applicable in the presence of invert sugar, but inapplicable in the presence of optically active non-sugars which change rotation with acidity.)

Prepare a normal solution or a solution of such fractional normality as the nature of the substance and the sensibility of the saccharimeter requires. Make the solution to volume at the temperature at which the polariscopic observations are to be made. Clarify with the minimum quantity of dry basic lead acetate. Products of higher purity may be clarified by adding alumina cream before making to volume. Filter.

151351°—20—5

(If desired, the excess of lead may be removed at this point. Add pulverized potassium or sodium oxalate to complete precipitation of lead. The deleading reagent should be added to the whole filtrate. If the deleading is omitted, the lead is satisfactorily removed by the chlorides subsequently added.)

Proceed as described under (a) or (b).

(a) Pipette two 70 ml (or 50 ml + 20 ml of water) portions of the clear filtrate into two 100 ml flasks. If preferred, 75 ml portions may be taken.

To one portion add 2.315 g of sodium chloride or 7.145 ml of a saturated sodium chloride solution or 10 ml of a solution containing 231.5 g per liter; make to volume at the temperature at which the observations are to be made and polarize.

To the other portion add hydrochloric acid and invert by one of the methods described on page 181. Cool and make to volume at the temperature at which the observations are to be made. Polarize.

(b) Pipette two 90.91 ml portions of the clear solution into two 100 ml flasks.

To one portion add 2.315 g of dry sodium chloride or 7.145 ml of saturated sodium chloride solution or fill to the mark with a solution of  $d_{4}^{20^{\circ}}$  1.164. Make to volume at the temperature at which the observations are to be made. Polarize.

Place the other portion in the water bath and heat to 65°. Add 5 ml of 38.8 per cent HCl ( $d_{4}^{20^{\circ}}$  1.1918, 42.96 Brix) or its exact equivalent of less concentrated acid and invert by method (f) on page 181. Convert all readings to the 26 g basis by multiplying by the factor:

$$\frac{\text{Volume of original solution containing 26 g of sample.}}{\text{Volume of solution taken for polarization.}}$$

From Table 19 find under the column which designates the volume taken for inversion the value of the Clerget divisor. Apply the temperature correction. If the original solution was of fractional normality,  $P - P'$  must be multiplied by this fraction before selecting the value from Table 19. Divide into  $P - P'$ .

See examples 1 and 2 under Methods I and II.

TABLE 19.—Method IV

[Sucrose + 2.315 g NaCl = +99°38 S; 13 g invert sugar  $\times$  2 = -33°25 S]

P - P'	Volume of solution taken for invert polarization				Temperature corrections (to be subtracted)											
	50 ml $\times$ 2	70 ml $\times \frac{10}{7}$	75 ml $\times \frac{4}{3}$	90.91 ml $\times \frac{11}{10}$												
133.35	-----	-----	-----	133.35	20.0	0.00	23.0	1.59	26.0	3.18	29.0	4.77	32.0	6.36		
133.07	-----	-----	133.07	-----	20.1	.05	23.1	1.64	26.1	3.23	29.1	4.82	32.1	6.41		
132.98	-----	132.98	-----	-----	20.2	.11	23.2	1.70	26.2	3.29	29.2	4.88	32.2	6.47		
132.63	132.63	-----	-----	-----	20.3	.16	23.3	1.75	26.3	3.34	29.3	4.93	32.3	6.52		
133	132.63	132.97	133.06	133.33	20.4	.21	23.4	1.80	26.4	3.39	29.4	4.98	32.4	6.57		
130	132.61	132.95	133.03	133.30	20.5	.27	23.5	1.86	26.5	3.44	29.5	5.04	32.5	6.63		
125	132.58	132.91	132.98	133.24	20.6	.32	23.6	1.91	26.6	3.50	29.6	5.09	32.6	6.68		
120	132.54	132.86	132.94	133.18	20.7	.37	23.7	1.96	26.7	3.55	29.7	5.14	32.7	6.73		
115	132.51	132.81	132.89	133.13	20.8	.42	23.8	2.01	26.8	3.60	29.8	5.19	32.8	6.78		
110	132.47	132.76	132.84	133.07	20.9	.48	23.9	2.07	26.9	3.66	29.9	5.25	32.9	6.84		
105	132.44	132.72	132.79	133.01	21.0	.53	24.0	2.12	27.0	3.71	30.0	5.30	33.0	6.89		
100	132.41	132.67	132.74	132.95	21.1	.58	24.1	2.17	27.1	3.76	30.1	5.35	33.1	6.94		
95	132.38	132.63	132.69	132.89	21.2	.64	24.2	2.23	27.2	3.82	30.2	5.41	33.2	7.00		
90	132.35	132.58	132.64	132.83	21.3	.69	24.3	2.28	27.3	3.87	30.3	5.46	33.3	7.05		
85	132.31	132.54	132.59	132.77	21.4	.74	24.4	2.33	27.4	3.92	30.4	5.51	33.4	7.10		
80	132.28	132.49	132.54	132.71	21.5	.80	24.5	2.39	27.5	3.98	30.5	5.57	33.5	7.16		
75	132.24	132.45	132.50	132.65	21.6	.85	24.6	2.44	27.6	4.03	30.6	5.62	33.6	7.21		
70	132.21	132.40	132.45	132.59	21.7	.90	24.7	2.49	27.7	4.08	30.7	5.67	33.7	7.26		
65	132.18	132.36	132.40	132.53	21.8	.95	24.8	2.54	27.8	4.13	30.8	5.72	33.8	7.31		
60	132.15	132.31	132.35	132.47	21.9	1.01	24.9	2.60	27.9	4.19	30.9	5.78	33.9	7.37		
55	132.11	132.26	132.30	132.41	22.0	1.06	25.0	2.65	28.0	4.24	31.0	5.83	34.0	7.42		
50	132.08	132.21	132.25	132.35	22.1	1.11	25.1	2.70	28.1	4.29	31.1	5.88	34.1	7.47		
45	132.04	132.17	132.20	132.29	22.2	1.17	25.2	2.76	28.2	4.35	31.2	5.94	34.2	7.53		
40	132.01	132.12	132.15	132.23	22.3	1.22	25.3	2.81	28.3	4.40	31.3	5.99	34.3	7.58		
35	131.98	132.08	132.10	132.17	22.4	1.27	25.4	2.86	28.4	4.45	31.4	6.04	34.4	7.63		
30	131.95	132.03	132.05	132.11	22.5	1.33	25.5	2.92	28.5	4.51	31.5	6.10	34.5	7.69		
25	131.92	131.99	132.00	132.05	22.6	1.38	25.6	2.97	28.6	4.56	31.6	6.15	34.6	7.74		
20	131.89	131.94	131.95	131.99	22.7	1.43	25.7	3.02	28.7	4.61	31.7	6.20	34.7	7.79		
15	131.85	131.89	131.90	131.93	22.8	1.48	25.8	3.07	28.8	4.66	31.8	6.25	34.8	7.84		
10	131.82	131.84	131.85	131.87	22.9	1.54	25.9	3.13	28.9	4.72	31.9	6.31	34.9	7.90		
5	131.78	131.79	131.80	131.81	23.0	1.59	26.0	3.18	29.0	4.77	32.0	6.36	35.0	7.95		

## 11. ANALYSES OF KNOWN MIXTURES

In Table 20 we have recorded data obtained in the analyses of sucrose mixtures of known composition. In every instance pure dry sucrose was weighed out before adding the impurity. The weight in 100 ml of the final solution was divided by 26.00 to obtain the per cent sucrose taken. The impurity was then added in varying amount.

In column 2 is indicated the analytical method employed. Analyses 2 and 4 were performed as described in method II, except that sodium chloride and sodium hydroxide were used instead of the corresponding ammonium salts. For the direct polarization

3.636 g of sodium chloride was added; the solution for invert polarization was neutralized after inversion with sodium hydroxide. The value of the divisor was found from equations (18), (22), (38) on page 177. The remaining analyses were done as described in the methods indicated.

TABLE 20.—Results of Analyses of Mixtures Containing Known Quantities of Sucrose

Experiment No.	Method	Impurity	P	P'	Temperature	Corrected divisor	Sucrose found	Sucrose taken
		Per cent	°S	°S	°C		Per cent	Per cent
1	IV—90.91 ml for P; 70 cc for P'...	0	99.34 <sub>6</sub>	—33.58 <sub>4</sub>	20.00	132.98	100.00	100.00
2	(II)—NaCl 90.91 ml for P; 70 ml for P'.....	0	98.93 <sub>7</sub>	—34.44 <sub>3</sub>	19.94	133.42	99.97	100.00
3	IV—90.91 ml for P and P'.....	<i>a</i> 20.4	72.92 <sub>8</sub>	—35.70 <sub>8</sub>	20.05	133.07	84.64	84.60
4	(II)—NaCl 70 ml for P and P'.....	<i>a</i> 20.4	76.59 <sub>6</sub>	—36.13 <sub>1</sub>	19.83	133.28	84.57	84.60
5	IV—90.91 ml for P and P'.....	<i>a</i> 13.9	29.95 <sub>6</sub>	—16.32 <sub>3</sub>	20.32	132.14	35.02	34.99
6	II—70 ml for P and P'.....	<i>a</i> 22.22	43.73 <sub>9</sub>	—24.72 <sub>3</sub>	20.24	132.99	51.47	51.41
7	II—70 ml for P and P'.....	<i>b</i> 30.76	90.71 <sub>3</sub>	—1.64 <sub>6</sub>	20.11	133.26	69.31	69.21
8	I—90.91 ml for P and P'.....	0	52.25 <sub>1</sub>	—17.35 <sub>3</sub>	20.14	133.14	52.28	52.28
9	IV—75 ml N/2 solution for P and P'.....	<i>c</i> 40.68	27.60	—15.92	20.10	131.91	32.99	33.08

*a* Invert sugar.

*b* Dextrose.

*c* 17.36 per cent invert, 23.32 per cent nonsugars from fermented molasses, making a synthetic "black strap."

In illustration of the inadequacy of the Clerget method when applied to mixtures of sucrose and invert sugar, Browne <sup>41</sup> performed five analyses on such mixtures, using his modification of Clerget's procedure. His analytical results reproduced directly from his article are assembled in Table 21. We have recalculated these data, using his corrected invert polarizations in unaltered form, but correcting his direct polarization to the values which he would have obtained if he had added sodium chloride to the solution to equalize the effect of the hydrochloric acid in the invert polarization. His solution for invert polarization contained 5 ml of 38.8 per cent hydrochloric acid in 55 ml, or 9.09 ml in 100 ml. We have shown on page 162 that 2.315 g of salt are required for the direct polarization if 5 ml of acid are contained in 100 ml of the invert polarization. Therefore, if 9.09 ml of acid are used,  $\frac{9.09}{5} \times 2.315 = 4.209$  g of sodium chloride should be contained in 100 ml of the solution for direct polarization. As we have shown on page 165, equation (22), this weight of salt dimin-

<sup>41</sup> J. Assoc. Off. Agr. Chem., II, pp. 138; 1916.



ishes the rotation of the normal solution of pure sucrose by  $0.265 \times 4.209 = 1.12$ . This quantity was deducted from the value which Browne used for his divisor <sup>42</sup>—viz,  $144.9 - 1.12 = 143.78$ —to obtain the basic value of his divisor if he had added 4.209 g of salt to his direct polarization.

The decrease of polarization of pure sucrose for the added salt is proportional to the quantity of sucrose present. We have, therefore, computed for each of his five analyses the decrease of rotation which the sucrose would have suffered if 4.209 g of salt had been added, by multiplying 1.12 by the per cent of sucrose taken. These corrections are given in column 3 of Table 22.

We have now to calculate the magnitude of the change of rotation which the invert sugar in the direct polarization would have undergone in the presence of the added salt. Thirteen grams of inverted sucrose in 100 ml of aqueous solution rotates  $-16.00$ .<sup>43</sup> We have from equation (18) on page 158 that twice the rotation of 13 g of inverted sucrose in the presence of 4.209 g of salt is  $-32.00 - (0.540 \times 4.209) = -34.27$  at  $20^\circ \text{C}$ . This, divided by 2, is  $-17.13$ . The addition of salt, then, changes the rotation of 13 g by  $-17.13 - (-16.00) = -1.13$ , or of 1 g by  $-0.087$ . The addition of salt would then have increased the negative rotation of the invert sugar in the direct polarization, and thus diminished the latter by 0.087 times the weight of invert sugar taken. These values are given in column 2 of Table 22. The combined effect of salt on the sucrose and on the invert sugar has been applied to Browne's observed direct polarization (column 3 of Table 21) to give the direct polarization which he would have obtained if he had added 4.209 g of salt to his direct polarization. These values are given in column 4 of Table 22. The divisor calculated from Browne's formula on page 129 corrected for the effect of salt on sucrose—namely,  $-1.12$ —is given in column 5. The essential agreement between the "sucrose taken" and "sucrose found," as shown in columns 6 and 7, indicates the adequacy of the corrective influence of the salt added to the solution for direct polarization.

<sup>42</sup> See p. 129.<sup>43</sup> See p. 167.

**TABLE 21.—Browne's Analyses on Sucrose, Invert Sugar Mixtures, Showing the Inadequacy of the Clerget Method**

Number	Invert sugar	Direct polarization (A)	Corrected invert polarization (B)	Temperature (t)	Sucrose (S)	
					Found	Taken
	G/100 ml			°C	Per cent	Per cent
1.....	1.10	94.60	—35.20	19.8	96.18	96.15
2.....	2.74	73.35	—30.42	20.0	77.10	76.92
3.....	5.48	50.70	—27.28	20.4	58.14	57.69
4.....	8.21	28.15	—24.04	20.4	38.99	38.46
5.....	9.86	6.90	—19.58	20.4	19.82	19.23

**TABLE 22.—Recalculation of Browne's Analyses to Show the Corrective Influence of Sodium Chloride in Equalizing the Rotation of Invert Sugar in the Direct and Invert Polarizations**

Number	Correction applied to direct polarization		Direct polarization corrected for effect of NaCl	Clerget divisor	Sucrose found	Sucrose taken
	Effect of NaCl on invert sugar	Effect of NaCl on sucrose				
1.....	—0.09	—1.08	93.43	133.82	96.12	96.15
2.....	— .24	— .86	72.25	133.46	76.93	76.92
3.....	— .48	— .65	49.58	133.01	57.78	57.69
4.....	— .71	— .43	27.01	132.74	38.46	38.46
5.....	— .86	— .21	5.83	132.49	19.18	19.23

The authors are indebted to Dr. C. A. Skinner, chief of the optical division, for a critical review of the manuscript.

### VIII. SUMMARY

1. The velocity of inversion of sucrose has been measured at temperatures from 20 to 90° C and has been found to follow the exponential law first proposed by Arrhenius. Rates of reaction were measured for three different concentrations of hydrochloric acid. The time required under different conditions to produce 99.99 per cent inversion was computed and tabulated.

2. The rate of decomposition of invert sugar in the presence of hydrochloric acid at different temperatures has been measured and the conclusion drawn that for the Clerget analysis 60° C is the most satisfactory temperature.

3. The value of the rotation, multiplied by 2, of 13 g of sucrose in 100 ml inverted and polarized in the presence of 6.34 N HCl was found to be, —33.25, at 20° C. Herzfeld's value, —32.66, was concluded to be in error. The basic value of the Clerget divisor was found to be 143.25 instead of 142.66.

4. The presence of hydrochloric acid increases the levo rotation of invert sugar. If  $C$  represents the number of milliliters of 6.34 HCl in 100 milliliters, the rotation  $R$  of invert sugar is

$$R_{T=20^{\circ}} = -32.00 - 0.125 C$$

5. Similar measurements were made with other reagents, and it was found that the effect of hydrochloric acid could be duplicated by neutral salts; 2.315 g of NaCl, or 2.451 g  $K_2C_2O_4$ , or 1.761 g  $CaCl_2$  produces the same effect as 10 milliliters of 6.34 N HCl. It is proposed to add 2.315 g of NaCl to the solution for direct polarization to secure a constant rotation of invert sugar when it exists as an impurity.

6. The effect of these reagents on the rotation of sucrose was measured.

7. The rotation of invert sugar in the presence of hydrochloric acid of varying concentration was measured and the conclusion drawn that in the method of inversion proposed no decomposition of invert sugar occurred during, or subsequent to, the reaction.

8. Various values of the rotation of invert sugar measured by other authors were found to be closely correlated by equation (16). This correlation extended from zero milliliter to 30 milliliter of 6.34 N HCl.

9. The value of the Clerget divisor in the presence of hydrochloric acid neutralized by NaOH and by  $NH_4OH$  was determined. This makes possible an analysis of sucrose in the presence of optically active substances which change in rotation with the hydrogen ion content of the solution.

10. The errors in the prevalent methods of analysis were tabulated. A convenient method of diluting to one-tenth greater volume by use of a 90.91 milliliter pipette is proposed.

11. Four methods of analytical procedure, each of particular application, were suggested and the values of the Clerget divisor tabulated in a convenient form.

12. Sucrose solutions of known composition were analyzed by the proposed methods. The analytical results were found to be accurate.

WASHINGTON, October 21, 1919.

NOTE.—During the typesetting of the foregoing manuscript a number of additional experiments have been performed which have corroborated the conclusions drawn. Many important questions are obviously in a state of uncertainty. Such questions as are incidental to the methods of clarification we have not attempted to study extensively. We are still in doubt in regard to the advisability of adding acetic

acid to the direct polarization in addition to the salt solution. If the filtrate from the basic lead clarification is basic, conceivably a basic lead chloride is precipitated which may occlude reducing sugars. Unquestionably an excess of acetic acid must be avoided, but there is much difficulty in determining how much constitutes an excess. If, however, the whole filtrate is dealed by means of an oxalate, the entire question is avoided.





